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# Using Ecological Niche Modeling and Genetics to Evaluate the Conservation Status of the Texas Gartersnake, *Thamnophis Sirtalis Annectens*

Kayla N. Key

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USING ECOLOGICAL NICHE MODELING AND GENETICS TO EVALUATE  
THE CONSERVATION STATUS OF THE TEXAS GARTERSNAKE,  
*THAMNOPHIS SIRTALIS ANNECTENS*

by

KAYLA N. KEY

A thesis submitted in partial fulfillment  
of the requirements for the degree  
of

Master of Science  
Department of Biology

John S. Placyk, Jr., Ph.D., Committee Chair  
College of Arts and Sciences

The University of Texas at Tyler  
May 2015

The University of Texas at Tyler  
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This is to certify that the Master's Thesis Dissertation of

KAYLA N. KEY

has been approved by the thesis requirement on  
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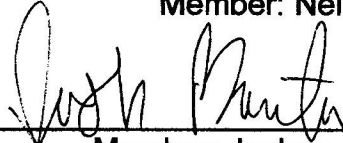
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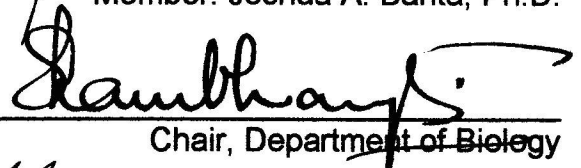
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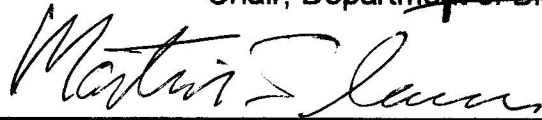
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## Abstract

### USING ECOLOGICAL NICHE MODELING AND GENETICS TO EVALUATE THE CONSERVATION STATUS OF THE TEXAS GARTERSNAKE, *THAMNOPHIS SIRTALIS ANNECTENS*

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The definition of a species has been argued extensively by philosophers and biologists resulting in the development of many different concepts which often contradict each other (Naomi, 2011). An integrative approach using multiple types of data (e.g, morphological, ecological, behavioral, genetic) may be the most successful at correctly assigning taxonomic levels (Pante et al., 2014). This approach may also be the best way to evaluate subspecific classifications (Makowsky et al., 2010). While many discount the existence of subspecies, the use of this level of classification is often vital in the conservation of populations (Haig et al., 2006). Here, we use an integrative approach of ecological niche modeling and molecular genetics to investigate the taxonomy of a state imperiled gartersnake subspecies, *Thamnophis sirtalis annectens*, using ecological niche modeling and molecular phylogenetics analyses. The distribution, taxonomy, population biology, and natural history, in general, of the Texas gartersnake (*Thamnophis sirtalis annectens*) are poorly known. It was described in the 1950's as one of the 12 currently recognized subspecies of the common gartersnake (*T. sirtalis*) based solely on morphological data. Since its initial description, its behavior, ecology, and systematics have not been examined and it remains one of the more enigmatic of the 12 subspecies. Recently, it was given a conservation rank of S2 (imperiled) in the

state of Texas and those that are familiar with it have suggested that its numbers are dwindling. Using ecological niche modeling and mtDNA sequence data we begin to understand the natural and evolutionary history of *T. s. annectens*. The results of this study provided additional information on the ecology and potential habitat range of *T. s. annectens* as well as information on the phylogenetic systematics of this subspecies. Our ecological niche model indicates areas where conservation efforts for *T. s. annectens* should be focused as well as important environmental variables such as landcover and geology that *T. s. annectens* prefers. When including *T. s. annectens* in a comparative niche model, this subspecies primarily occupies distinctly different habitat than the red-sided gartersnake, *T. s. parietalis*, which also occurs in Texas. Statistical analysis indicated that *T. s. annectens* occupies a significantly different ecological niche than *T. s. parietalis*. Similarly, the genetic data indicate that *T. s. annectens* can be differentiated from *T. s. parietalis* and *T. s. sirtalis*, however this difference is greatest between *T. s. sirtalis*. While this work has told us much about *T. s. annectens*, more is left to be learned including ground-truthing our ecological niche model. Collecting additional genetic data to verify the phylogenetic relationships we have hypothesized here should also be done in the future. Regardless, this work indicates *T. s. annectens* may be distinct both genetically and ecologically and provides conservation managers with niche models that will assist in locating the optimal habitat required by this subspecies.

## Chapter One

# Using Ecological Niche Modeling and Genetics to Evaluate the Conservation Status of the Texas gartersnake, *Thamnophis sirtalis annectens*

## Introduction

Philosophers and biologists have spent a significant amount of time searching for the "best" species concept (Naomi, 2011). This search has resulted in over 20 different definitions of what a species is (Frankham et al., 2012). These concepts are usually a product of different subgroups of biologists advocating for the concept that concurs with their areas of expertise and organisms that they study (Naomi, 2011; de Queiroz, 2007). Many of the different species concepts do not agree with each other resulting in much taxonomic confusion (de Queiroz, 2007). This is an obvious problem when trying to delineate species (de Queiroz, 2007). The three most widely used species concepts are the biological species concept (BSC), the evolutionary species concept (ESC), and the phylogenetic species concept (PSC) (Frankham et al., 2012). The biological species concept (BSC) defines species as "groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups" (Mayr, 1942). The BSC remains the most widely accepted concept (Singh, 2012), but it does have significant downfalls. For instance, the BSC ignores situations in which speciation is incomplete making the concept non-evolutionary (Singh, 2012). This is an issue for many biologists who feel that the definition or concept of a species must involve evolutionary criteria. The evolutionary species concept (ESC) states that a species is a "lineage of ancestral descent which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate" (Simpson, 1951). Mayr rejected the evolutionary species concept on the ground that the ESC was a definition of

a phyletic lineage and not of a species (Singh, 2012). The phylogenetic species concept defines a species as "the smallest diagnosable cluster of individual organisms with which there is a parental pattern of ancestry and descent" (Cracraft, 1983). Frankham et al. (2012) suggest the major problem with the phylogenetic species concept is that with the advances in technology, making DNA sequencing more affordable, it may lead to more detailed information among lineages. Because of the lack of limitation on what is to be a "small cluster", individuals within a population may be considered a different lineage under the PSC. A fourth species concept, the ecological species concept, introduced a new property into species concepts, the ecological niche (Van Valen, 1976). A species under the ecological species concept is "a lineage (or closely related set of lineages) which occupies an adaptive zone minimally different from that of any other lineage in its range and which evolves separately from all lineages outside its range" (Van Valen, 1976). This concept is based on the idea that species are lineages that share the same niche. An issue that could arise with this concept would be instances of local adaptation that could be confused with the same lineage plastically occupying different ecological niches. However, localized phenotypes may eventually undergo genetic assimilation in which the local phenotype would then be genetically encoded and thus be recognized as a "new" species under many different concepts (Pigliucci et al., 2006; Placyk, 2011).

Although there are major differences between these concepts, there is an underlying property that they all have in common: species are "separately evolving metapopulation lineages" (de Queiroz, 2007). Concepts differ in the "secondary" defining properties of a species which are unique to each concept (de Queiroz, 2007). de Queiroz (2007) suggests that the differences in secondary properties among species concepts comes from where the species "line" is drawn during the process of speciation. A unified species concept has been put forth by de Queiroz (2007), which is based on

the original common property that previous species concepts are based on except there are no secondary properties of species specifically listed in this concept. Instead, all secondary properties are considered "lines of evidence" and are used to assess the separation of lineages (de Queiroz, 2007).

An integrative approach using multiple lines of evidence such as differences in morphology, behavior, life-history traits, ecology, physiology, and genetics may be most successful for correctly assigning taxonomic levels. This approach may be the best way to evaluate subspecific levels as well. As discussed earlier, there are also problems or discrepancies of definitions of subspecies concepts. Some of the issues with subspecies concepts stem from the original confusion of species concepts (e.g., which lines of evidence are to be considered). Originally the term "subspecies" was used to replace the term "varieties" (Torstrom et al., 2014). Mayr (1963) defines subspecies as "an aggregate of local populations of a species, inhabiting a geographic subdivision of the range of the species, and differing taxonomically from other populations of the species." The ambiguity of "differing taxonomically" has been the major issue with the subspecies definition because it can be interpreted multiple ways (morphologically, genetically, behaviorally, etc.) (Torstrom et al., 2014). In the past, subspecies were described based on morphological and geographical differences alone (Makowsky et al., 2010). Recently, critics of this past method of subspecies identification suggest that the differing morphological traits in which subspecies were first described may not reflect underlying genetic and phylogenetic structure (Haig et al., 2006). Because of this issue, there has been a need to standardize what differing taxonomically means and quantify differences using statistical measures such as the 75% rule (Torstrom et al., 2014). This rule helps to delimitate subspecies if 75% of the population exhibits morphological traits that are different from other populations (Torstrom et al., 2014). However there have been issues with this rule in that some suggest that the threshold of 75% is not high enough

and that it should be raised to 95% in accordance to the standard 0.05 of other statistical tests (Tortstrom et al., 2014). Another issue is that phenotypic plasticity could be driving the morphological differences rather than genetic differences.

As Tortstrom et al (2014) points out, the "molecular revolution" has infiltrated the description process of subspecies. Now, molecular phylogenetic-based taxonomy is incorporated in describing subspecies or dissolving subspecies by sequencing mitochondrial or nuclear genes to calculate genetic divergence between currently existing subspecies (Tortstrom et al., 2014). If two subspecies have a high genetic divergence, then they would remain two separate subspecies or possibly be elevated to species level. If two subspecies have a low genetic divergence, then the subspecies status would dissolve. When morphologically described subspecies are reexamined using molecular genetics, there tends to be inconsistencies in what should be a considered a subspecies (Makowsky et al., 2010). The inconsistencies of subspecies concepts, despite the criticism of the subspecies taxa in general, can have extensive impacts in taxonomy and conservation which is why it is important to acknowledge these inconsistencies and provide an integrative "solution".

Subspecies are recognized and listed for protection under the U.S. Endangered Species Act of 1973 (ESA), the World Conservation Union (IUCN) Red List of Threatened Species, TRAFFIC (wildlife trade monitoring network), Canada's Species at Risk Act, and many others (Haig et al., 2006). It is important that conservation efforts are not being wasted on subspecies that are not in fact unique. The relatively recent inconsistencies of naming subspecies have caused difficulty in conservation efforts by misdirecting those efforts. This has been shown with notable cases of morphologically described subspecies that did not "hold true" when genetically analyzed (Phillimore and Owens, 2005). One notable case of morphologically based subspecies determination includes the now extinct dusky seaside sparrow (*A. m. nigrescens*). In 1966, the dusky



seaside sparrow was listed as "endangered" under the ESA (Awise and Nelson, 1980). By 1980, there were only 6 male individuals that could be found and 5 were brought into captivity to become a part of a captive breeding program (Awise and Nelson, 1980). The breeding program consisted of breeding these males with a morphologically similar subspecies from the Gulf coast populations. This breeding program was not federally funded as it was said that the intergrades would not be protected under the ESA because they would not be true duskies (Rhymer and Simberloff, 1996). The breeding program was unsuccessful, and the last dusky seaside sparrow died in captivity in 1987 (Awise and Nelson, 1980). Later Awise and Nelson (1989) examined mtDNA gene sequences of the dusky seaside sparrow in comparison to 9 other subspecies from both the Gulf coast populations and the Atlantic coast populations. They reported that there was no evidence to suggest that the dusky seaside sparrow was phylogenetically distinct from other Atlantic coast populations of *A. martinus* meaning these populations should have been used in the breeding program instead of the Gulf coast populations (Awise and Nelson, 1980). This story emphasizes the importance of integrative approaches, in this case morphology and genetic analyses, in informing and designing conservation strategies.

Accurately delineating subspecies of conservation concern is extremely important to conservation efforts and success. Not only are morphological, geographical, and molecular differences essential in making this delineation, but ecological differences are important as well. Given the conflicting information provided by morphological and geographical versus genetic data, some have found that the use of molecular phylogenetic analyses along with ecological niche modeling has helped to untangle taxonomic uncertainty (Raxworthy et al., 2007; Rissler and Apodaca, 2007). Subspecies delineation of Day Geckos in Madagascar has been successful using morphology, mtDNA, and ecological niche modeling (Raxworthy et al., 2007). The results of this study

suggested the elevation of three subspecies of *Phelsuma madagascariensis*, and the description of a new species *Phelsuma ravenala*. Although *Phelsuma ravenala* had low levels of molecular divergence with its sister species *Phelsuma dubia*, it did exhibit ecological niche differences and morphologically diagnostic characters (Raxworthy et al., 2007). Phylogeography and ecological niche modeling were used to investigate the Black Salamander (*Aneides flavipunctatus*), a species with low levels of morphological variation (Rissler and Apodaca, 2007). These results suggested the presence of two distinct lineages within this species. Using mtDNA evidence and ecological niche modeling, Rissler and Apodaca (2007) suggested that these two lineages be elevated to species status. These studies represent the need for an integrative approach in evaluating subspecies taxa and how this approach can be used to successfully delimit subspecies. I have taken an integrative approach here to investigate three subspecies of the common gartersnake, *Thamnophis sirtalis*, using ecological niche modeling and phylogenetic analyses, where one of these three subspecies, the Texas gartersnake, is of conservation interest.

The common gartersnake, *Thamnophis sirtalis*, is known for its extensive geographic distribution that ranges farther north than any other snake species in the Western Hemisphere and from the Atlantic Coast to the Pacific Coast of the USA (Rossman et al., 1996). As a result, *T. sirtalis* occurring across most of the USA along with high abundance in many parts of its range, it is one of the most thoroughly studied species of snake in the world. One reason that *T. sirtalis* may be so successful is its generalist nature and its ability to adjust to its environment via phenotypic plasticity and local adaptations (Placyk, 2011). Both help it to occupy many different habitats (e.g., margins of ponds, upland fallow fields, mountain ranges, urban areas) (Rossman et al., 1996). *Thamnophis sirtalis* is also a generalist in its selection of prey items, feeding on a variety of prey including invertebrates, fish, amphibians and their larvae, reptiles

(including other snakes) and mammals (Rossman et al., 1996). Given the wide distribution of *T. sirtalis*, it often displays morphological characters that differ from one geographic range to the next. This polymorphism has resulted in 12 subspecies being described (Crother et al., 2012) (Table 1).

Although there is much information on the behavior, physiology, evolution, ecology, and life-history of *Thamnophis sirtalis*, as a species, information on specific subspecies is often vague or non-existent. For example, since its initial description in 1950, no further work has been conducted to verify the taxonomic status of the Texas gartersnake, *T. s. annectens*. Rather, all work on *T. s. annectens* published after 1950 has primarily focused on updating the range of this subspecies (Gutberlet et al., 1998; Dixon, 2000; Lardie, 2001) or brief overviews of current knowledge on natural history (Rossman et al., 1996; Tennant, 2003). However, despite these few range updates, those who are familiar with this subspecies suggest that there is still a need for updated verification of its geographic range, and Dixon (2000) states that it needs reexamination taxonomically.

The Texas gartersnake was initially described in 1950 based on morphology and geographic distribution that differed from the other two subspecies that occur in Texas, the red-sided gartersnake, *T. s. parietalis*, and the eastern gartersnake, *T. s. sirtalis* (Brown, 1950). The historic distribution of *T. s. annectens* ranges from east-central Texas through west-central Oklahoma to the eastern Texas panhandle and southwestern Kansas (Brown, 1950; Gutberlet et al., 1998; Dixon, 2000; Lardie, 2001; Tennant, 2003) (Figure 1). This range is met and sometimes overlaps with historic distribution of both *T. s. parietalis* in Oklahoma and in the Texas Panhandle and *T. s. sirtalis* in east Texas (Rossman et al., 1996) (Figure 1). However, there is some confusion about range boundaries between *T. s. annectens* and the other two subspecies in Texas and Oklahoma, and intergrades between *T. s. annectens* and the

other two subspecies have been observed (Brown, 1950; Gutberlet et al., 1998; Dixon, 2000; Lardie, 2001) (Figure 1). Populations of *T. s. annectens* are believed to have been most common historically in the original tall-grass prairies of Texas and Oklahoma, which were characterized, in part, by small wetlands that provided habitat for anurans, a food source for *T. s. annectens* (Tennant, 2003). However, in recent decades these prairies have been disturbed and often completely destroyed by conversion to agricultural areas (Tennant, 2003). In addition to tall-grass prairies, *T. s. annectens* has been observed in woodland and riparian bottomland habitat (Tennant, 2003). Currently, we know very little about the evolution, ecology, and behavior of *T. s. annectens*. To compound this paucity of data, *T. s. annectens* is also considered a species of conservation concern in the state of Texas, being recently listed as state imperiled (Texas Park and Wildlife Department, 2012).

Recent molecular work suggests that the subspecific status of several of the currently recognized subspecies of the common gartersnake, *T. sirtalis*, are either not warranted (e.g., the Chicago gartersnake, *T. s. semifasciatus*; Placyk et al., 2007) or masks what may actually be considered full species (e. g., the maritime gartersnake, *T. s. pallidulus*; Placyk and Galvan, unpublished data). In a molecular phylogeographic study on *T. sirtalis* subspecies that occur in western North America, none of the morphologically-based subspecies in the study region were supported by mtDNA analyses (Janzen et al., 2002). They found in multiple subspecies that some populations were actually more closely genetically related to populations of other subspecies than populations of the same subspecies. Placyk et al. (2007) found greater levels of sequence divergence (up to 7.7%) within a single subspecies of *T. s. sirtalis* as compared to Janzen et al. (2002) who sampled four subspecies with only up to 2.5% divergence between any given pair of subspecies. This is surprising in that the

subspecies Janzen et al. (2002) evaluated were extremely different morphologically. In a similar story, *T. s. annectens* is considered more morphologically similar to *T. s. sirtalis* than *T. s. parietalis*, but seems to have more potential range overlap with *T. s. parietalis* than *T. s. sirtalis*. Given the uncertainty of the status of *T. sirtalis* as a group, the general lack of natural history data, and possible declines of *T. s. annectens*, detailed studies on this subspecies are crucial.

This study aimed to (a) determine specific habitat requirements and the potential distribution of *T. s. annectens* using ecological niche modeling (b) compare ecological niche models of *T. s. annectens* with two other subspecies that occur in Texas and (c) conduct a phylogenetic analysis to be compared to ecological differences that may occur (shown via comparative niche models).

## Chapter Two

### Methods

#### Ecological Niche Modeling Methods

Ecological niche models determine relationships between known locations of species and predetermined environmental variables (i.e., ArcGIS layers). The models then project this relationship across a landscape to provide probabilities of habitat suitability. The maximum entropy modeling method (MaxEnt) was applied to generate separate ecological niche models (ENMs) for *T. s. annectens*, *T. s. parietalis*, and *T. s. sirtalis*. This method uses incomplete information (i. e. presence only data) to find the probability distribution of maximum entropy (i. e. closest to uniform) given constraints of known locations and environmental variables (Raxworthy et al., 2007). I applied the software Maxent version 3.3.3k (Phillips et al., 2006; <http://www.cs.princeton.edu/~schapire/Maxent>) which generates a map showing probabilities of habitat suitability for each area of the landscape (given the spatial grain size) with values ranging from 0 to 1, where 0 is the most unsuitable areas and 1 is the most suitable areas.

The extent was restricted to the states of Oklahoma, Texas, Arkansas, and Louisiana which includes the historic distribution of *T. s. annectens* (Oklahoma and Texas). Arkansas and Louisiana were included to increase the number of presence points for *T. s. sirtalis*. Presence records were obtained for *T. s. annectens*, *T. s. parietalis*, and *T. s. sirtalis* via museum records, inaturalist records ([www.inaturalist.org](http://www.inaturalist.org)), and personal communications (Table 2). Occurrence points for *T. s. annectens* range in date from 2007-2014 while dates for the other two subspecies range from 1970-2014 (due to lack of available data). Presence points were projected to NAD 1983 UTM Zone

14N in ArcGIS 10.3. Six environmental GIS layers (Table 3) were used in the model for each subspecies: geology, landcover, mean temperature of coldest quarter, precipitation of driest quarter, mean temperature of wettest quarter, and mean temperature of driest quarter. All continuous environmental variables used in the model had a correlation of < 0.75 which reduces the effect of spatial autocorrelation. If layers were not in raster form when obtained, they were converted to raster from vector form in ArcMap 10.3. All environmental raster files were projected to NAD 1983 UTM Zone 14N and resampled as necessary to 1km x 1km spatial resolution. Layers were then clipped to the extent of the study area and converted into ASCII files to be used in Maxent. Selections in Maxent included an analysis to assess environmental variable contribution in making the habitat suitability map. Run type was set to cross-validate to generate test data, and random seed was chosen to randomize test data. To mitigate spatial autocorrelation, we randomly removed all but one occurrence record falling within the same grid cell as one another. All other settings in Maxent were set to default. Because Maxent does not require absence points, 10,000 pseudo-absence points are generated automatically.

To determine if the model was a good fit, the AUC value of each model was considered. The area under the curve the receiver operating curve (AUC) measures the probability that presence points will have a higher habitat suitability score than the randomly chosen pseudo-absence points (Phillips and Dudik, 2008). Test AUC values are based off of "test data" which are presence points that are partitioned out of the dataset used to create the model that are then used to "test" the model; specifically one point was set aside each run. Models with AUC and Test AUC values greater than 0.75 are considered usable models that provide sufficiently more predictive power than a random map (Elith, 2002). Maxent also reports gain, which represents the mean log probability of occurrence samples minus a constant that makes the uniform distribution

have zero gain (Phillips, 2006). We used the test gain values to assess which environmental variables were the most important for model fit.

The "average habitat suitability" map for each subspecies was converted into a binary map in ArcGIS 10.3 using the equal test sensitivity and specificity logistic threshold. This threshold allows for equal chance of error for false positives and false negatives (Phillips, 2006). All three binary maps were then combined using spatial analyst tools in ArcGIS 10.3 to create a comparative niche modeling map visually showing potential niche overlap (Raxworthy et al., 2007).

To test whether the habitat suitability maps for each subspecies were significantly different from each other, we used a program called ENMtools v 1.3 (Warren et al., 2010). Ecological niche models for each subspecies were used in the "measure niche overlap" module. This module conducts a pairwise comparison for all models and outputs three statistics, Schoener's D (Schoener, 1968), the *I* statistic (Warren et al., 2008), and relative rank (RR, Warren and Seifert, 2011). All three statistics range from 0 (species have completely different ecological niche models) to 1 (species have identical ecological niche models). Schoener's D was the statistic used in this study as Schoener's D, the *I* statistic, and RR are highly correlated (Warren et al., 2011). The empirically derived Schoener's D statistic was then compared for statistical significance against a null distribution created using a permutation test with 100 replicates (Pike, 2013). This test pools the occurrence points for two species, using randomly selecting points to create niche models, and then calculate overlap statistics. By comparing the observed Schoener's D (created in "Measure of niche overlap") to the null distribution Schoener's D (created in "Identity test"), one can test whether two species exhibit statistically significant ecological niches (Warren et al., 2011). We used



the five percent quantile of the permuted distribution to assess significance (Dunithan, 2012).

## **Molecular Ecology Methods**

All tissue samples were acquired through museum collections and collaborators (Table 4 & 5). All specimens used for this study were from Texas or the states immediately surrounding Texas (Table 4 & 5). If subspecies were not indicated, specimens were assigned subspecies based on location data (Dixon, 2000; Rossman et al., 1996). Total genomic DNA was extracted with the illustra™ tissue & cells genomicPrep Mini Spin Kit (Martin, 2013).

The entire ~1010 base pair gene of the NADH dehydrogenase 2 (ND2) gene were PCR-amplified using the forward primer L4437b (5'-CAG CTA AAA AAG CTA TCG GGC CCA TAC C-3'; Kumazawa et al., 1996), which lies in the tRNA-Met upstream of ND2, and the reverse primer Sn-ND2r (5'-GGC TTT GAA GGC TMC TAG TTT-3'; R. Lawson, pers. comm.), which lies in the tRNA-Trp downstream of ND2 (Placyk et al., 2007). For the cytochrome c oxidase I (COI) region, 695 base pairs were PCR-amplified using the forward primer FishF2\_t1 (5'-TGT AAA ACG ACG GCC AGT CGA CTA ATC ATA AAG ATA TCG GCA C-3'; Ivanova, et al., 2007) and the reverse primer FishR2\_t1 (5'- CAG GAA ACA GCT ATG ACA CTT CAG GGT GAC CGA AGA ATC AGA A-3'; Ivanova, et al., 2007). Polymerase chain reaction (PCR) protocols for ND2 and COI was conducted in 20- $\mu$ L volumes with 3.6 $\mu$ L RNase-free H<sub>2</sub>O, 10.0 $\mu$ L TopTaq Master Mix (Qiagen), 2.0 $\mu$ L Coral Load (Qiagen), 1.0 $\mu$ L each 2- $\mu$ mol primer, and 2.4 $\mu$ L DNA (Placyk, 2013). A negative control was included for all PCRs. The following parameters were used for ND2 mtDNA amplification: 30 cycles each consisting of 1 min of denaturing at 94 °C, 1 min of primer annealing at 55 °C, and 1.5 min of extension at 72 °C. The following parameters were used for COI mtDNA amplification: 2 min of

denaturing at 94°C, 30 s of primer annealing at 50°C, 35 cycles of 1 min of extension at 72°C, and a final extension of 10 min at 72°C.

Amplification of PCR product was verified using gel electrophoresis. PCR products were purified prior to sequencing with the E.Z.N.A. Cycle Pure Kit (OMEGA biotek). Sequencing reactions for ND2 were carried out using the primers L4437b (5'-CAG CTA AAA AAG CTA TCG GGC CCA TAC C-3') and L5238 (5'-ACM TGA CAA AAA ATY GC-3') (de Queiroz et al., 2002). Sequencing reactions for COI were carried out using the forward and reverse primer used in PCR amplification. Purified DNA was concentrated to the level recommended by Eurofins MWG Operon (20–40 ng/μL) and shipped to Eurofins MWG Operon where DNA was sequenced on an ABI 3730xl DNA sequencer. Sequences were manually proofread and edited using the program Sequencher 5.2.4 (Gene Codes Corporation, Ann Arbor, MI). Alignments were performed using Clustal X (Thompson et al., 1997) and a final round of editing and inter- and intra- subspecific divergence rate calculations for both genes were completed in Mesquite 3.01 (Maddison and Maddison, 2014). PhyML 3.1 was used to generate a ML tree for the ND2 gene (Guindon et al., 2010). Non-parametric bootstrap resampling was used to quantify the statistical support for the ML phylogeny using 1000 bootstrap replicates (Felsenstein, 1985). JModelTest 0.1 was used to determine the substitution model by using the Akaike Information Criterion corrected for small sample size (Posada, 2008). JModelTest determined HKY + I was the best model of sequence evolution for the data. The substitution model we used was the default HKY85 model in PhyML with proportion of invariable sites (I) estimated from the dataset. To root the ML tree we included a sequence from *T. proximus* (Alfaro and Arnold, 2001; GenBank access No. AF383847). This outgroup was chosen based on broad-scale phylogenetic analyses of *Thamnophis* phylogeny that show *T. proximus* as sister to *T. sirtalis* (Alfaro and Arnold, 2001).

## Chapter Three

### Results

#### Ecological Niche Modeling Results

The training AUC values of the ecological niche models were above 0.75. The test AUC values for *T. s. annectens* and *T. s. parietalis* were 0.89 and 0.92, respectively (Figures 4 & 13), whereas the test AUC for *T. s. sirtalis* was 0.741 (Figure 22). This lower test AUC for *T. s. sirtalis* could be due to the generalist nature of this subspecies, since more widespread species tend to have lower AUC values when modeled (Gonzalez et al., 2011). As a result, it should be noted that the niche model for *T. s. sirtalis* shows areas of high habitat suitability outside of its natural range (Figure 1) which could overestimate the overlap when compared with *T. s. annectens*. The relative contribution of environmental variables to niche models varied depending on subspecies (Figures 5, 14, & 23). For *T. s. annectens* and *T. s. sirtalis*, the most important environmental factor to the gain of the model was landcover (Figures 5 & 23). The top most suitable landcover type for *T. s. annectens* was "western great plains floodplain system" (0.86) and "east-central Texas plains post oak savannah and woodland" (0.76)(Figure 7 & Table 6). The top most suitable landcover type for *T. s. sirtalis* was "western great plains sand prairie" (0.94) as well as "west gulf coastal plain large river floodplain" (0.80)(Figure 25 & Table 6). For *T. s. parietalis*, the most important environmental layer in the model was mean temperature of driest quarter with the optimal range of  $-1.43^{\circ}\text{C}$  through  $1.453^{\circ}\text{C}$  (Figure 21 & Table 6).

The comparative niche model map indicated overlap in suitable habitat between *T. s. annectens* and the other two subspecies (Figure 31). Despite these areas of overlap, there tends to be isolated areas of high habitat suitability for each individual subspecies from the other subspecies (Figure 3). The ecological niche models of *T. s.*

*parietalis* and *T. s. annectens* show a low level of overlap and have significantly different niches, while *T. s. annectens* and *T. s. sirtalis* do not have significantly different ecological niches, *Thamnophis s. parietalis* and *T. s. sirtalis* are not considered to have significantly different ecological niches (Table 7).

## Molecular Ecology Results

We successfully sequenced the 1010 bp of the ND2 gene from 6 *T. s. annectens*, 4 *T. s. parietalis*, 10 *T. s. sirtalis*, and 2 individuals that may have been *T. s. parietalis/T. s. annectens* intergrades based on their collection location (Table 4). We also successfully sequenced 695 bp of COI gene for 3 *T. s. annectens*, 1 *T. s. parietalis*, 2 *T. s. sirtalis*, and 1 individual that may have been a *T. s. parietalis/T. s. annectens* intergrades based on its collection location (Table 5). Divergence estimates for the ND2 gene were as follows for each subspecies pairing: 0.048 or 4.8% between *T. s. sirtalis* and *T. s. parietalis*; 0.049 or 4.9% between *T. s. sirtalis* and *T. s. annectens*; 0.008 or 0.8% between *T. s. parietalis* and *T. s. annectens* (Table 10). Intrasubspecific variation was particularly high for *T. s. sirtalis* in the ND2 gene while intrasubspecific variation in *T. s. annectens* and *T. s. parietalis* were relatively low (Table 11). Divergence estimates for COI gene were lower than ND2 gene estimates with pairings as follows: 0.03 or 3.0% for *T. s. sirtalis* and *T. s. parietalis*; 0.031 or 3.1% for *T. s. sirtalis* and *T. s. annectens*; 0% for *T. s. parietalis* and *T. s. annectens*. Intrasubspecific variation was low for each subspecies for the COI gene (i.e. less than 1.0%) (Table 11). A rooted maximum likelihood tree graphically representing the relatedness of these three subspecies supports the divergence data showing *T. s. sirtalis* to be more distantly related to *T. s. annectens* and *T. s. parietalis*, while the latter two subspecies appear to be more closely related (Figure 34). In addition to these more clearcut distinctions between subspecies, our tree also indicates that two *T. s. sirtalis* (STX14 & STX16) are more closely related to

our *T. s. parietalis* and *T. s. annectens* individuals than to other *T. s. sirtalis* (Figure 34). We can find *T. s. annectens* in two separate clades, one in which there are only *T. s. annectens* grouped together (Figure 34). Both clades that include *T. s. annectens* are placed more closely to *T. s. parietalis* than to *T. s. sirtalis* (Figure 34).

## Chapter Four

### Discussion

#### Ecological Niche Modeling

The ecological niche modeling results provide insight as to which areas are important to *T. s. annectens* as well as what environmental variables determine its optimal habitat. The individual model for *T. s. annectens* shows areas of high suitability within the central Texas area and the eastern portion of the Texas panhandle (Figure 4). The areas suitable for *T. s. annectens* in central Texas are consistent with the historic range of the subspecies (Figure 1). However, within our model, there is an emphasis on the eastern Texas panhandle that is not represented in historic county records (Figure 2). Personal communications resulted in 33 occurrence points from the northeastern portion of the panhandle suggesting that there may be a viable population of *T. s. annectens* in that region of Texas. It may be that this population has historically existed but was not documented until now. Alternatively, it may indicate a range shift or expansion northwest for *T. s. annectens* (Figure 32). Unfortunately, there are no earlier records to determine which situation is the case.

Historically, *T. s. annectens* has been most abundant in tall grass prairie habitat such as the Blackland Prairie (Tennant, 2003), but our model suggests that the Great Plains Flood Plain System and East-central Texas plains oak savannah and woodlands may now be more suitable habitat (Figure 8). *Thamnophis sirtalis*, as a species, is known to be a generalist making feasible a possible shift in habitat feasible. The Blackland Prairie and East-central Texas plains post oak savannah border each other making it possible for remnant populations of *T. s. annectens* to migrate east into

suitable habitat in the post oak savannah habitat after degradation of the blackland prairie habitat. This information will be useful for conservation as it will provide more insight on what specific habitat types *T. s. annectens* prefers and in turn where conservation efforts should be focused (Figure 8). In the comparative niche model, it is quite visually obvious that *T. s. annectens* is ecologically differentiated from the other two subspecies of *T. sirtalis* that occur in Texas with only a few areas of potential overlap with the two other subspecies (Figure 31). Statistical analysis indicated *T. s. annectens* and *T. s. parietalis* occupy different ecological niches which provides evidence that *T. s. annectens* is playing a different ecological role than *T. s. parietalis*. Ecological niche differences between *T. s. parietalis* and *T. s. annectens* are mostly attributed to areas habitat suitability that differ in mean temperature throughout specific quarters of the year (coldest, wettest, and driest quarters) (Table 6). During the coldest quarter, areas with slightly warmer mean temperatures are more suitable for *T. s. annectens* than *T. s. parietalis* (Table 6). During the wettest quarter, the *T. s. parietalis* model indicates no particular temperature is important to habitat suitability while the *T. s. annectens* model shows a temperature of 23.8°C is important to habitat suitability (Table 6). During the driest quarter, *T. s. annectens* model indicates that a slightly higher temperature is important to habitat suitability than *T. s. parietalis* models (Table 6). Given these differences in temperature importance to habitat suitability, it seems that *T. s. parietalis* suitable habitat is found in areas with cooler temperature during specific quarters of the year while *T. s. annectens* suitable habitat is more likely to be in places of warmer temperature. Landcover type importance of the *T. s. annectens* model is highest with the West Great Plains Floodplain system which was the second highest landcover type of *T. s. parietalis* (Table 6). The lack of significant ecological niche difference between *T. s. sirtalis* and the other two subspecies could be related to the model quality of this subspecies. While the Test AUC was near the "good fit" model threshold, the

generalist nature of *T. s. sirtalis* could have made it difficult for Maxent to determine a pattern across the landscape. As a result, we see many areas of high habitat suitability outside of the historic range of *T. s. sirtalis* including some that extend into the far western parts of Texas (Figure 31). These "false-positive" areas may be masking an actual significant niche difference between *T. s. sirtalis* and the other two subspecies.

Future directions with this aspect of this study include ground-truthing the habitat suitability map for *T. s. annectens* and the other two subspecies which will provide us with more location data that can be used to improve the ecological niche model for this subspecies. The addition of occurrence points to the *T. s. sirtalis* may help more accurately predict areas of high habitat suitability within the actual range of this subspecies. Improved models could then be used to statistically reexamine differences in the ecological niches of these subspecies. Significant ecological differences could be used as lines of evidence in delineating between these subspecies. In addition, historic ecological niche models should be conducted in order to identify past important habitat types. These results compared with the current niche modeling results could give more information on the potential shift in habitat preference as well as population decline of *T. s. annectens* in tall grass prairies.

## **Molecular Ecology**

Our genetic data indicate several important features of the evolutionary history of the Texas gartersnake (*T. s. annectens*). Most importantly, *T. s. annectens* can be genetically differentiated from one of the two subspecies that occur within its historic range. Is this differentiation enough to warrant considering it to be a separate species? No. The divergence estimates between *T. s. parietalis*, and *T. s. annectens* is extremely low (i.e., less than 1.0%) indicating, if nothing else, that *T. s. parietalis* and *T. s.*



*annectens* are genetically subspecies of the same species. At the same time, however, both *T. s. parietalis* and *T. s. annectens* are ~4.75% divergent from *T. s. sirtalis* for the ND2 gene, which is extremely high given that Burbrink et al. (2000) elevated two subspecies of the ratsnake, *Pantherophis obsoleta*, to full species status based on 2.83-4.37% divergence. Torstrom et al. (2014) examined the literature focusing on Class Reptilia to examine any pattern of elevating or collapsing subspecies based on genetic distance values. He found that the median genetic distances used for elevating subspecies to species level was (6.4%), and the midpoint genetic distance ranged from 1.0% to 19.4% (Torstrom et al., 2014). The median genetic distance for collapsing subspecies was 1.0% (Torstrom et al., 2014). Torstrom et al. (2014) concluded that while there is not a universal genetic distance threshold for elevating or collapsing subspecies, there is a consensus that higher genetic distance is necessary for elevating a subspecies, and that these decisions should be done on a case-by-case bases with the phylogeny of closely related species considered (Torstrom et al., 2014). Our divergence rates between *T. s. sirtalis* and the other two subspecies fall within the midpoint genetic distance range for elevating subspecies.

Discussions with the Burbrink lab, who are currently attempting to complete a range-wide phylogeographic study of *T. sirtalis*, indicate that they are finding all subspecies of *T. sirtalis* that occur in Texas to be one and the same species, but our data might suggest otherwise. Our next step for this component of the study is to generate more additional sequence data for these three subspecies to validate our current findings.

Three less clearcut results are also evident from our phylogeny. Two involve unusual groupings of individuals from our data set. The first includes the individuals APTX57 and APTX58 (Figure 34), which form a clade sister with our other *T. s. parietalis*

and *T. s. annectens* individuals. These were individuals whose locations appeared to be a mix of *T. s. parietalis* and *T. s. annectens* ranges. We believe that these two individuals are *T. s. annectens/T. s. parietalis* intergrades and this is supported by their collection locales (Table 4), as both were collected where the historic range of *T. s. annectens* and *T. s. parietalis* merge in Oklahoma. Therefore, my genetic results support earlier reports of potential intergradation between *T. s. parietalis* and *T. s. annectens* (Brown, 1950; Gutberlet et al., 1998; Dixon, 2000; Lardie, 2001). Additional genetic data in the form of nuclear markers need to be collected to fully verify this as well as possibly examining the morphological characteristics of these two particular specimens. The second less clearcut result is the placement of what was thought to be two *T. s. sirtalis* (STX14 and STX16) in the *T. s. parietalis/ T. s. annectens* clade (Figure 34). We believe this to be a simple case of misidentification, as *T. sirtalis* is known to exhibit a very wide range of morphological features throughout its range (e.g., Rossman et al. 1996) with different subspecies easily being confused for each other where they overlap. Note that the two *T. s. sirtalis* (STX14 and STX16) that are most closely related to *T. s. parietalis* and *T. s. annectens* individuals are also both from Arkansas counties (Table 4; Figure 3) close to the Oklahoma border which is near the range boundaries of both *T. s. parietalis* and *T. s. sirtalis* (Figure 35). Therefore, we believe this was simply a misidentified *T. s. parietalis*. The intrasubspecific variation within *T. s. sirtalis* is reduced by 3.0% when these two individuals are removed from the analysis (Table 11). When STX 14 & 16 are excluded in the intersubspecific divergence analysis, rates are still above 4.65% for each pairing of *T. s. sirtalis* with the other two subspecies (Table 10). Changes in intrasubspecific divergence of *T. s. sirtalis* and the lack of change in intersubspecific divergence seems to support that these two individuals (STX 14 & 16) are *T. s. parietalis*.

The third less clearcut result involves three individuals (ATX12, ATX13 and ATX18) thought to be *T. s. annectens* (Table 4) based on range data (Rossman et al., 1996). These three individuals are from counties in far west Oklahoma (Figure 35) close to the Texas panhandle. We believe that these individuals are indeed *T. s. annectens*. All other *T. s. annectens* individuals were grouped within the larger *T. s. parietalis* clade suggesting that these *T. s. annectens*, from the central part of its range, may not be genetically different from *T. s. parietalis* (Figure 34). When the three *T. s. annectens* individuals (ATX12, ATX13 and ATX18) are treated as the only "true" *T. s. annectens* group, the intersubspecific sequence divergence rate for ND2 between *T. s. parietalis* and *T. s. annectens* is still < 1%. Despite the low sequence divergence rate, we believe the grouping we see with three *T. s. annectens* individuals is indicating the beginning of a divergence from *T. s. parietalis* (Figure 34). Future investigation into this population of *T. s. annectens* and the populations in the Texas panhandle may provide more detail on this divergence from *T. s. parietalis*.

## Chapter Five

### Conclusions

Our results from both ecological niche modeling and molecular ecology complement each other and indicate that *T. s. annectens* is in fact unique when compared to the other two subspecies of *T. sirtalis* that occur in Texas. Differences in habitat requirements via niche models show that suitable *T. s. annectens* habitat occupies areas that differ from both *T. s. sirtalis* and *T. s. annectens*. Although *T. s. annectens* and *T. s. parietalis* were statistically not shown to have different niches from *T. s. sirtalis*, both show high genetic divergence from *T. s. sirtalis* (Table 9; Figure 34). This genetic differentiation may be due, in part, to a lack of sympatry throughout much of the ranges of the three subspecies in Texas as evidenced by our niche models (Figure 31). *Thamnophis s. annectens* and *T. s. parietalis* are shown here to have a different ecological niche, although they are extremely similar genetically (Table 9; Figure 34). These results are even more peculiar when the distinctive coloration difference between these two subspecies is considered (Rossman et al., 1996). This ecological difference may have started as phenotypic plasticity of *T. s. annectens* in which over time resulted in niche evolution, or it could be that these two subspecies are simply in the process of speciation in which mitochondrial DNA has not fully yet diverged (Figure 34) (Placyk, 2011). Given the ecological and genetic differences, *T. s. annectens* appears to be different from the two other subspecies of *T. sirtalis* that occur in Texas. This study provides another example of how an integrative approach using multiple lines of evidence can be useful in investigating taxonomy. In particular, ecological niche modeling and phylogenetic analyses has been used here to investigate a subspecies of conservation concern. By using multiple lines of evidence, we have a clearer picture of

how these subspecies of *T. sirtalis* differ. Further investigation into each of these aspects, as well as into the morphology, behavior, and life-history of the three subspecies in Texas will provide more detailed information about exactly how different *T. s. annectens* is from the other two subspecies.

## Conservation Implications

The results of this study provide additional information on the ecology and potential habitat range of *T. s. annectens* as well as an examination of the phylogenetic systematics of this subspecies (Figures 31 & 34). Our ecological niche model indicates areas where conservation efforts for *T. s. annectens* should be focused (Figures 4 & 8) as opposed to depending on less contemporary and possibly no longer pertinent historic data (Figure 32). We also have provided a county-based map showing current potential range for *T. s. annectens* (Figure 33). Given that *T. s. annectens* is of conservation concern, our results provide an important conclusion: *T. s. annectens* is ecologically and genetically unique when compared to *T. s. parietalis* and *T. s. sirtalis*. While the differences in each of these aspects are not enough on their own to elevate *T. s. annectens* to a species level, together both ecological niche modeling and genetics provide enough information to consider this subspecies unique which can be used in further conservation efforts. In the mean time, we would strongly suggest that *T. s. annectens* continue to be listed as state imperiled or possibly even elevated to state threatened in Texas.

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## Appendix

Table 1. Subspecies of *Thamnophis sirtalis* (Crother et al., 2012).

Species	Common name
<i>T. s. annectens</i>	Texas gartersnake
<i>T. s. concinnus</i>	Red-spotted gartersnake
<i>T. s. dorsalis</i>	New Mexico gartersnake
<i>T. s. fitchi</i>	Valley gartersnake
<i>T. s. infernalis</i>	California red-sided gartersnake
<i>T. s. pallidulus</i>	Maritime gartersnake
<i>T. s. parietalis</i>	Red-sided gartersnake
<i>T. s. pickeringii</i>	Puget Sound gartersnake
<i>T. s. semifasciatus</i>	Chicago gartersnake
<i>T. s. similis</i>	Blue-striped gartersnake
<i>T. s. sirtalis</i>	Eastern gartersnake
<i>T. s. tetrataenia</i>	San Francisco gartersnake

Table 2. Presence points of *Thamnophis* subspecies and sources used for ecological niche modeling.

Species	Latitude	Longitude	Source
<i>T. s. annectens</i>	35.91452	-100.29860	Richard Kazmaier
<i>T. s. annectens</i>	35.91147	-100.29714	Richard Kazmaier
<i>T. s. annectens</i>	35.91549	-100.29394	Richard Kazmaier
<i>T. s. annectens</i>	35.91219	-100.29241	Richard Kazmaier
<i>T. s. annectens</i>	35.90150	-100.29107	Richard Kazmaier
<i>T. s. annectens</i>	35.91374	-100.29125	Richard Kazmaier
<i>T. s. annectens</i>	35.91375	-100.29125	Richard Kazmaier
<i>T. s. annectens</i>	35.91370	-100.29108	Richard Kazmaier
<i>T. s. annectens</i>	35.91376	-100.29107	Richard Kazmaier
<i>T. s. annectens</i>	35.91368	-100.29099	Richard Kazmaier
<i>T. s. annectens</i>	35.91035	-100.28947	Richard Kazmaier
<i>T. s. annectens</i>	35.90337	-100.28895	Richard Kazmaier
<i>T. s. annectens</i>	35.90265	-100.28868	Richard Kazmaier
<i>T. s. annectens</i>	35.90326	-100.28816	Richard Kazmaier
<i>T. s. annectens</i>	35.90248	-100.28676	Richard Kazmaier
<i>T. s. annectens</i>	35.90246	-100.28558	Richard Kazmaier
<i>T. s. annectens</i>	35.90248	-100.28554	Richard Kazmaier
<i>T. s. annectens</i>	35.90217	-100.28182	Richard Kazmaier
<i>T. s. annectens</i>	35.90199	-100.28123	Richard Kazmaier
<i>T. s. annectens</i>	35.90184	-100.28083	Richard Kazmaier
<i>T. s. annectens</i>	35.90199	-100.27961	Richard Kazmaier
<i>T. s. annectens</i>	35.90144	-100.27956	Richard Kazmaier
<i>T. s. annectens</i>	35.90131	-100.27899	Richard Kazmaier
<i>T. s. annectens</i>	35.90438	-100.27832	Richard Kazmaier
<i>T. s. annectens</i>	35.90181	-100.27781	Richard Kazmaier
<i>T. s. annectens</i>	35.90194	-100.27750	Richard Kazmaier
<i>T. s. annectens</i>	35.90200	-100.27744	Richard Kazmaier
<i>T. s. annectens</i>	35.90201	-100.27742	Richard Kazmaier
<i>T. s. annectens</i>	35.91600	-100.27614	Richard Kazmaier
<i>T. s. annectens</i>	35.89531	-100.26808	Richard Kazmaier
<i>T. s. annectens</i>	35.89806	-100.26623	Richard Kazmaier
<i>T. s. annectens</i>	35.89386	-100.26424	Richard Kazmaier
<i>T. s. annectens</i>	35.89036	-100.20406	Richard Kazmaier
<i>T. s. annectens</i>	32.18483	-97.09975	Clint King
<i>T. s. annectens</i>	32.68390	-96.92091	Mike Malevich
<i>T. s. annectens</i>	32.64763	-97.64871	Mark Pyle
<i>T. s. annectens</i>	30.36003	-97.64740	John Williams
<i>T. s. annectens</i>	32.73005	-96.75250	inaturalist
<i>T. s. annectens</i>	32.86588	-96.73370	inaturalist
<i>T. s. annectens</i>	33.27015	-95.90115	Jerrold Tynes
<i>T. s. annectens</i>	33.27745	-95.30466	Jerrold Tynes
<i>T. s. annectens</i>	33.26907	-95.90034	Jerrold Tynes
<i>T. s. annectens</i>	32.71200	-97.47800	Mark Pyle
<i>T. s. annectens</i>	32.95000	-96.43080	Lani Lyman- Henley
<i>T. s. parietalis</i>	36.91452	-96.15620	Carnegie Museum of Natural History
<i>T. s. parietalis</i>	36.62228	-94.86720	MVZ Herp Catalog
<i>T. s. parietalis</i>	36.74898	-98.13940	Carnegie Museum of Natural History

<i>T. s. parietalis</i>	36.81123	-98.13290	Carnegie Museum of Natural History
<i>T. s. parietalis</i>	36.61608	-100.10900	Univ. of Colorado Museum of Nat. History
<i>T. s. parietalis</i>	34.12470	-94.67080	Smithsonian Institution
<i>T. s. parietalis</i>	36.86998	-97.69030	University of Kansas Biodiversity Institute
<i>T. s. parietalis</i>	36.95555	-96.55140	University of Kansas Biodiversity Institute
<i>T. s. parietalis</i>	36.19492	-95.63480	Carnegie Museum of Natural History
<i>T. s. sirtalis</i>	36.62228	-99.85097	Carnegie Museum of Natural History
<i>T. s. sirtalis</i>	36.54578	-99.55501	Carnegie Museum of Natural History
<i>T. s. sirtalis</i>	36.60103	-99.38514	Carnegie Museum of Natural History
<i>T. s. sirtalis</i>	34.69000	-94.13639	Smithsonian Institution
<i>T. s. sirtalis</i>	31.61140	-93.40080	Univ. of Colorado Museum of Nat. History
<i>T. s. sirtalis</i>	29.00107	-95.81584	Katy Snakes

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Table 3. The environmental layers used for ecological niche modeling of *Thamnophis* subspecies.

Environmental variables	Source
Geology	USGS
Landcover	USGS
Bio 11: Mean temperature of coldest quarter	WorldClim
Bio 17: Precipitation of driest quarter	WorldClim
Bio 8: Mean temperature of wettest quarter	WorldClim
Bio 9: Mean temperature of driest quarter	WorldClim

Table 4. Tissue sample localities of *Thamnophis* subspecies and sources used in ND2 gene analyses.

Subspecies	ID	State	County/Parish	City	Source ID	Source
<i>T. s. annectens</i>	ATX1	Texas	Rockwall			L. Lyman
<i>T. s. annectens</i>	ATX2	Texas	Rockwall			L. Lyman
<i>T. s. sirtalis</i>	STX6	Louisiana	Plaquemines	Belle Chasse	CAS 207052	CAS
<i>T. s. sirtalis</i>	STX7	Louisiana	St. John the Baptist	La Place	CAS 207053	CAS
<i>T. s. parietalis</i>	PTX8	Oklahoma	Muskogee	Muskogee	OMNH 6690	OMNH
<i>T. s. parietalis</i>	PTX9	Oklahoma	Muskogee	Muskogee	OMNH 6694	OMNH
<i>T. s. parietalis</i>	PTX10	Oklahoma	Muskogee	Muskogee	OMNH 6749	OMNH
<i>T. s. annectens</i>	ATX12	Oklahoma	Ellis	Arnett	OMNH 7063	OMNH
<i>T. s. annectens</i>	ATX13	Oklahoma	Ellis	Arnett	OMNH 7108	OMNH
<i>T. s. sirtalis</i>	STX14	Arkansas	Montgomery	Norman	OMNH 2838	OMNH
<i>T. s. parietalis</i>	PTX15	Oklahoma	Le Flore	Sallisaw	OMNH 2474	OMNH
<i>T. s. sirtalis</i>	STX16	Arkansas	Scott	Poteau mtn	OMNH 2526	OMNH
<i>T. s. annectens</i>	ATX18	Oklahoma	Roger Mills	Durham	OMNH 2664	OMNH
<i>T. s. sirtalis</i>	STX26	Louisiana	East Feliciana		LSUMZ H-1823	LSUMZ
<i>T. s. sirtalis</i>	STX27	Louisiana	East Baton Rouge		LSUMZ H-2024	LSUMZ
<i>T. s. sirtalis</i>	STX28	Louisiana	East Baton Rouge		LSUMZ H-2025	LSUMZ
<i>T. s. sirtalis</i>	STX29	Louisiana	Iberville		LSUMZ H-2081	LSUMZ
<i>T. s. annectens</i>	ATX47	Texas	McLennan		LSUMZ H-7775	LSUMZ
<i>T. s. sirtalis</i>	STX54	Louisiana	Jefferson		LSUMZ H-8772	LSUMZ
<i>T. s. annectens/parietalis</i>	APT57	Oklahoma	Cleveland	Norman	LSUMZ H-9326	LSUMZ
<i>T. s. annectens/parietalis</i>	APT58	Oklahoma	Cleveland	Norman	LSUMZ H-9327	LSUMZ
<i>T. s. sirtalis</i>	STX67	Louisiana	Saint Martin		LSUMZ H-20419	LSUMZ

(CAS- California Academy of Science; OMNH-Sam Noble Oklahoma Museum of Natural History; LSU-Louisiana Museum of Natural History)

Table 5. Tissue sample localities of *Thamnophis* subspecies and sources used in COI gene analyses.

Subspecies	ID	State	County/Parish	City	Source ID	Source
<i>T. s. annectens</i>	ATX1	Texas	Rockwall			L. Lyman
<i>T. s. annectens</i>	ATX2	Texas	Rockwall			L. Lyman
<i>T. s. annectens</i>	ATX21	Texas	Dallas	Dallas		S. Christman
<i>T. s. annectens/parietalis</i>	APT57	Oklahoma	Cleveland	Norman	LSUMZ H-9326	LSUMZ
<i>T. s. parietalis</i>	PTX10	Oklahoma	Muskogee	Muskogee	OMNH 6749	OMNH
<i>T. s. sirtalis</i>	STX29	Louisiana	Iberville		LSUMZ H-2081	LSUMZ
<i>T. s. sirtalis</i>	STX7	Louisiana	St. John the Baptist	La Place	CAS 207053	CAS

(CAS- California Academy of Science; OMNH-Sam Noble Oklahoma Museum of Natural History; LSU-Louisiana Museum of Natural History)



Table 6. Results from response curves of environmental layers for each subspecies showing the optimal values within each layer. Numbers in parentheses indicate the probability of presence for each species at the given value.

Environmental Layer	<i>T. s. annectens</i>	<i>T. s. parietalis</i>	<i>T. s. sirtalis</i>
Geology	Terrace (0.669)	Water (0.725)	Sand (0.605)
		Shale (0.663)	
Landcover	Western great plains floodplain system (0.856)	Southeastern great plain tall grass prairie (0.807)	Western great plains sand prairie (0.943)
	East-central Texas plains post oak savannah and woodland (0.763)	Western great plains floodplain system (0.78)	Western gulf coastal plain large river floodplain (0.795)
		Water (0.77)	
Mean temperature of coldest quarter	3.15°C (0.714)	-0.219°C through 1.6°C (0.804)	-0.23°C through 1.275°C (0.514)
Precipitation of driest quarter	42.87mm (0.658)	0mm to 7.43mm (0.524)	All values were equal (0.50)
Mean temperature of wettest quarter	23.834°C (0.637)	All values were equal (0.50)	All values were equal (0.50)
Mean temperature of driest quarter	3.136°C (0.661)	-1.43°C through 1.45°C (0.771)	-1.43°C through 1.25°C (0.51)

Table 7. Comparison of observed Schoener's D to 5 percent quantile of Schoener's D of the null distribution.

Subspecies Comparison	Observed Schoener's D	5% of Null Schoener's D
<i>T. s. annectens</i> X <i>T. s. parietalis</i>	0.38	0.49
<i>T. s. annectens</i> X <i>T. s. sirtalis</i>	0.46	0.29
<i>T. s. parietalis</i> X <i>T. s. sirtalis</i>	0.38	0.33

Table 8. Unique geological characteristic of rock type with corresponding ID value shown in response curves produced by Maxent.

VALUE	Primary Rock Type
1	sand
2	evaporite
3	clay or mud
4	sandstone
5	shale
6	water
7	terrace
8	mixed clastic/carbonate
9	fine-grained mixed clastic
10	mudstone
11	limestone
12	silt
13	gravel
14	alluvial fan
15	dolostone (dolomite)
16	basalt
17	playa
18	landslide
19	quartzite
20	granite
21	rhyolite
22	conglomerate
23	siltstone
24	indeterminate
25	trachyte
26	granodiorite
27	phyllite
28	paragneiss
29	amphibole schist
30	coarse-grained mixed clastic
31	gneiss
32	diorite
33	claystone
34	serpentine
35	medium-grained mixed clastic
36	chert
37	tuff
38	novaculite
39	ash-flow tuff
40	alluvium
41	gabbro
42	carbonate
43	alluvial terrace
44	dune sand
45	clastic
46	loess
47	alkalic intrusive rock



Table 9. Unique landcover type with corresponding ID value shown in response curves produced by Maxent.

VALUE	Landcover Category
1	West Gulf Coastal Plain Upland Longleaf Pine Forest and Woodland
2	Central and South Texas Coastal Fringe Forest and Woodland
3	East Gulf Coastal Plain Maritime Forest
4	East Gulf Coastal Plain Southern Loess Bluff Forest
5	East Gulf Coastal Plain Southern Mesic Slope Forest
6	Mississippi Delta Maritime Forest
7	West Gulf Coastal Plain Chenier and Upper Texas Coastal Fringe Forest and Woodland
8	West Gulf Coastal Plain Mesic Hardwood Forest
9	East-Central Texas Plains Pine Forest and Woodland
10	West Gulf Coastal Plain Pine-Hardwood Forest
11	West Gulf Coastal Plain Sandhill Oak and Shortleaf Pine Forest and Woodland
12	East Gulf Coastal Plain Interior Upland Longleaf Pine Woodland - Loblolly Modifier
13	East Gulf Coastal Plain Interior Upland Longleaf Pine Woodland - Offsite Hardwood Modifier
14	East Gulf Coastal Plain Near-Coast Pine Flatwoods - Offsite Hardwood Modifier
15	Evergreen Plantation or Managed Pine
16	Madrean Encinal
17	Madrean Pinyon-Juniper Woodland
18	Madrean Pine-Oak Forest and Woodland
19	Madrean Upper Montane Conifer-Oak Forest and Woodland
20	Edwards Plateau Dry-Mesic Slope Forest and Woodland
21	Edwards Plateau Limestone Savanna and Woodland
22	Edwards Plateau Mesic Canyon
23	Llano Uplift Acidic Forest, Woodland and Glade
24	Edwards Plateau Limestone Shrubland
25	Crosstimbers Oak Forest and Woodland
26	East-Central Texas Plains Post Oak Savanna and Woodland
27	Lower Mississippi River Dune Woodland and Forest
28	Mississippi River Alluvial Plain Dry-Mesic Loess Slope Forest
29	Crowley's Ridge Sand Forest
30	Ouachita Montane Oak Forest
31	Ozark-Ouachita Dry Oak Woodland
32	Ozark-Ouachita Dry-Mesic Oak Forest
33	Managed Tree Plantation
34	Ruderal forest
35	Atlantic Coastal Plain Fall-line Sandhills Longleaf Pine Woodland - Offsite Hardwood
36	East Gulf Coastal Plain Interior Shortleaf Pine-Oak Forest - Hardwood Modifier
37	East Gulf Coastal Plain Interior Shortleaf Pine-Oak Forest - Mixed Modifier
38	Ozark-Ouachita Shortleaf Pine-Bluestem Woodland
39	Ozark-Ouachita Shortleaf Pine-Oak Forest and Woodland
40	East Gulf Coastal Plain Northern Mesic Hardwood Forest

41	Ozark-Ouachita Mesic Hardwood Forest
42	Crowley's Ridge Mesic Loess Slope Forest
43	Rocky Mountain Subalpine Dry-Mesic Spruce-Fir Forest and Woodland
44	Southern Rocky Mountain Juniper Woodland and Savanna
45	Southern Rocky Mountain Pinyon-Juniper Woodland
46	Western Great Plains Floodplain
47	Western Great Plains Floodplain Systems
48	Central Interior and Appalachian Floodplain Systems
49	Central Interior and Appalachian Riparian Systems
50	Ozark-Ouachita Riparian
51	South-Central Interior Large Floodplain
52	South-Central Interior / Upper Coastal Plain Wet Flatwoods
53	East Gulf Coastal Plain Large River Floodplain Forest - Forest Modifier
54	East Gulf Coastal Plain Small Stream and River Floodplain Forest
55	East Gulf Coastal Plain Tidal Wooded Swamp
56	East-Central Texas Plains Floodplain Forest
57	Southeastern Great Plains Riparian Forest
58	Southeastern Great Plains Floodplain Forest
59	Mississippi River Bottomland Depression
60	Mississippi River Floodplain and Riparian Forest
61	Mississippi River Low Floodplain (Bottomland) Forest
62	Mississippi River Riparian Forest
63	Red River Large Floodplain Forest
64	Southern Coastal Plain Blackwater River Floodplain Forest
65	West Gulf Coastal Plain Large River Floodplain Forest
66	West Gulf Coastal Plain Near-Coast Large River Swamp
67	West Gulf Coastal Plain Small Stream and River Forest
68	Gulf and Atlantic Coastal Plain Swamp Systems
69	West Gulf Coastal Plain Seepage Swamp and Baygall
70	East Gulf Coastal Plain Southern Loblolly-Hardwood Flatwoods
71	Lower Mississippi River Flatwoods
72	Southern Coastal Plain Nonriverine Basin Swamp
73	West Gulf Coastal Plain Nonriverine Wet Hardwood Flatwoods
74	West Gulf Coastal Plain Pine-Hardwood Flatwoods
75	Edwards Plateau Riparian
76	East Gulf Coastal Plain Near-Coast Pine Flatwoods
77	East Gulf Coastal Plain Near-Coast Pine Flatwoods - Open Understory Modifier
78	West Gulf Coastal Plain Wet Longleaf Pine Savanna and Flatwoods
79	Rocky Mountain Montane Riparian Systems
80	North American Warm Desert Lower Montane Riparian Woodland and Shrubland
81	North American Warm Desert Riparian Systems
82	North American Warm Desert Riparian Woodland and Shrubland
83	Tamaulipan Floodplain
84	Tamaulipan Riparian Systems
85	Rocky Mountain Gambel Oak-Mixed Montane Shrubland
86	Rocky Mountain Lower Montane-Foothill Shrubland
87	Central Mixedgrass Prairie
88	Western Great Plains Foothill and Piedmont Grassland
89	Western Great Plains Tallgrass Prairie

90	Western Great Plains Sand Prairie
91	Western Great Plains Sandhill Steppe
92	Western Great Plains Mesquite Woodland and Shrubland
93	Western Great Plains Shortgrass Prairie
94	Arkansas Valley Prairie and Woodland
95	Southeastern Great Plains Tallgrass Prairie
96	Texas Blackland Tallgrass Prairie
97	Texas-Louisiana Coastal Prairie
98	Central Interior Highlands Calcareous Glade and Barrens
99	Coahuilan Chaparral
100	Mogollon Chaparral
101	West Gulf Coastal Plain Catahoula Barrens
102	West Gulf Coastal Plain Nepheline Syenite Glade
103	West Gulf Coastal Plain Northern Calcareous Prairie
104	West Gulf Coastal Plain Southern Calcareous Prairie
105	Central and Upper Texas Coast Dune and Coastal Grassland
106	South Texas Dune and Coastal Grassland
107	South Texas Sand Sheet Grassland
108	Texas Coastal Bend Beach
109	Upper Texas Coast Beach
110	East Gulf Coastal Plain Savanna and Wet Prairie
111	Texas-Louisiana Coastal Prairie Slough
112	Eastern Great Plains Wet Meadow, Prairie and Marsh
113	Western Great Plains Depressional Wetland Systems
114	Chihuahuan-Sonoran Desert Bottomland and Swale Grassland
115	Gulf and Atlantic Coastal Plain Tidal Marsh Systems
116	Mississippi Sound Salt and Brackish Tidal Marsh
117	Texas Saline Coastal Prairie
118	North American Warm Desert Playa
119	Apacherian-Chihuahuan Mesquite Upland Scrub
120	Apacherian-Chihuahuan Semi-Desert Grassland and Steppe
121	Chihuahuan Creosotebush, Mixed Desert and Thorn Scrub
122	Chihuahuan Gypsophilous Grassland and Steppe
123	Chihuahuan Loamy Plains Desert Grassland
124	Chihuahuan Mixed Desert and Thorn Scrub
125	Chihuahuan Sandy Plains Semi-Desert Grassland
126	Chihuahuan Stabilized Coppice Dune and Sand Flat Scrub
127	Chihuahuan Succulent Desert Scrub
128	Madrean Juniper Savanna
129	North American Warm Desert Active and Stabilized Dune
130	Chihuahuan Mixed Salt Desert Scrub
131	Sonora-Mojave Mixed Salt Desert Scrub
132	South Texas Lomas
133	Tamaulipan Calcareous Thornscrub
134	Tamaulipan Clay Grassland
135	Tamaulipan Mesquite Upland Scrub
136	Tamaulipan Mixed Deciduous Thornscrub
137	Tamaulipan Savanna Grassland
138	Colorado Plateau Mixed Low Sagebrush Shrubland
139	Inter-Mountain Basins Semi-Desert Shrub Steppe
140	East Gulf Coastal Plain Large River Floodplain Forest - Herbaceous Modifier

141	Rocky Mountain Cliff, Canyon and Massive Bedrock
142	Western Great Plains Badland
143	Southwestern Great Plains Canyon
144	Western Great Plains Cliff and Outcrop
145	North American Warm Desert Badland
146	North American Warm Desert Bedrock Cliff and Outcrop
147	North American Warm Desert Pavement
148	Rocky Mountain Alpine Bedrock and Scree
149	Unconsolidated Shore
150	Undifferentiated Barren Land
151	Cultivated Cropland
152	Pasture/Hay
153	Introduced Upland Vegetation - Annual Grassland
154	Introduced Upland Vegetation - Perennial Grassland and Forbland
155	Modified/Managed Southern Tall Grassland
156	Introduced Riparian and Wetland Vegetation
157	Disturbed, Non-specific
158	Harvested Forest - Grass/Forb Regeneration
159	Harvested Forest-Shrub Regeneration
160	Recently burned grassland
161	Recently burned shrubland
162	Disturbed/Successional - Grass/Forb Regeneration
163	Disturbed/Successional - Shrub Regeneration
164	Open Water (Brackish/Salt)
165	Open Water (Fresh)
166	Quarries, Mines, Gravel Pits and Oil Wells
167	Developed, Open Space
168	Developed, Low Intensity
169	Developed, Medium Intensity
170	Developed, High Intensity

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Table 10. Intersubspecific divergence rates (%) for both ND2 and COI mitochondrial genes for three subspecies of *Thamnophis sirtalis*

Subspecies X Subspecies	ND2	COI
<i>T. s. annectens</i> X <i>T. s. parietalis</i>	0.79%	0%
<i>T. s. annectens</i> X <i>T. s. sirtalis</i>	4.85%	3.02%
<i>T. s. annectens</i> X <i>T. s. sirtalis</i> **	4.75%**	N/A**
<i>T. s. parietalis</i> X <i>T. s. sirtalis</i>	4.75%	3.02%
<i>T. s. parietalis</i> X <i>T. s. sirtalis</i> **	4.65%**	N/A**

\*\*When STX 14& 16 excluded

Table 11. Intrasubspecific divergence rates (%) for both ND2 and COI mitochondrial genes for three subspecies of *Thamnophis sirtalis*

Subspecies	ND2	COI
<i>T. s. annectens</i>	0.49%	0%
<i>T. s. parietalis</i>	0.29%	N/A*
<i>T. s. sirtalis</i>	4.55%	0.57%
<i>T. s. sirtalis</i> **	1.55%**	N/A**

\*one sample used; \*\*When STX 14& 16 excluded

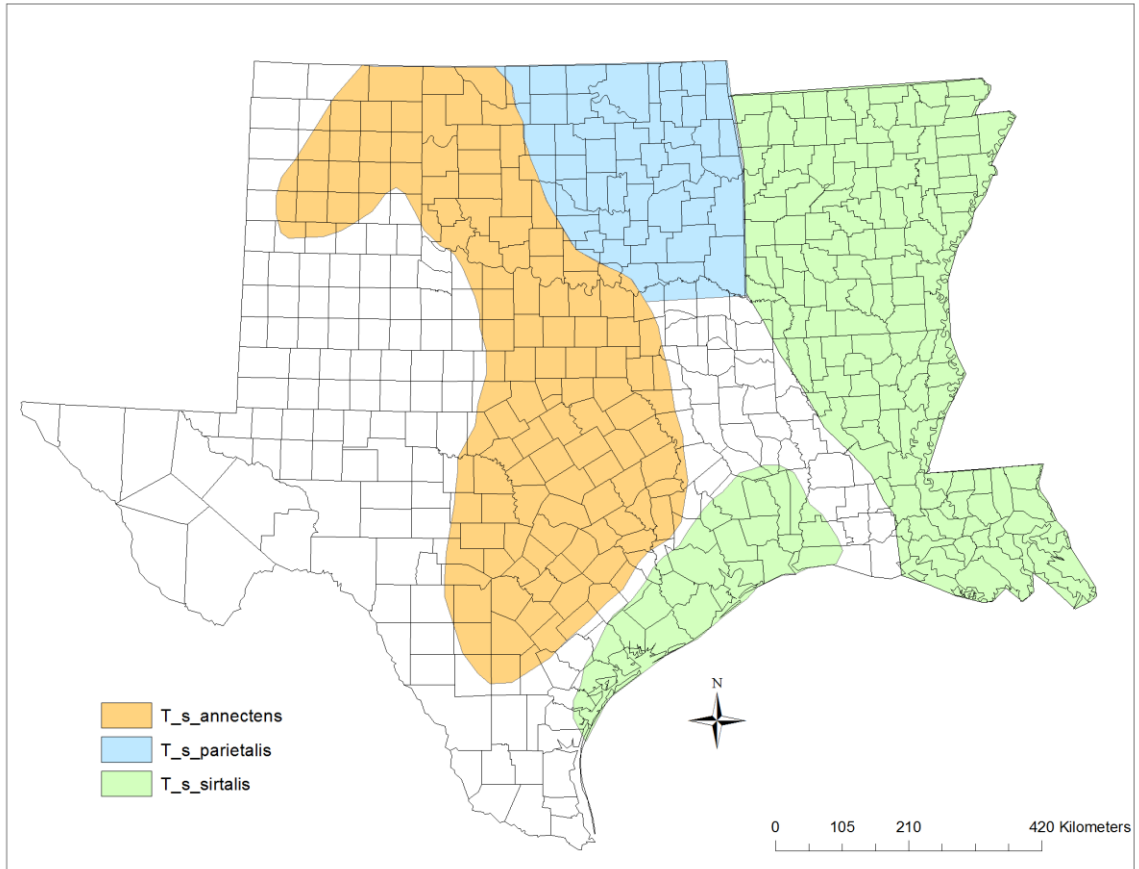


Figure 1. Historic ranges of the Texas gartersnake (*T. s. annectens*), the red-sided gartersnake (*T. s. parietalis*), and the eastern gartersnake (*T. s. sirtalis*) in Texas and neighboring states. (Rossman et al., 1996).

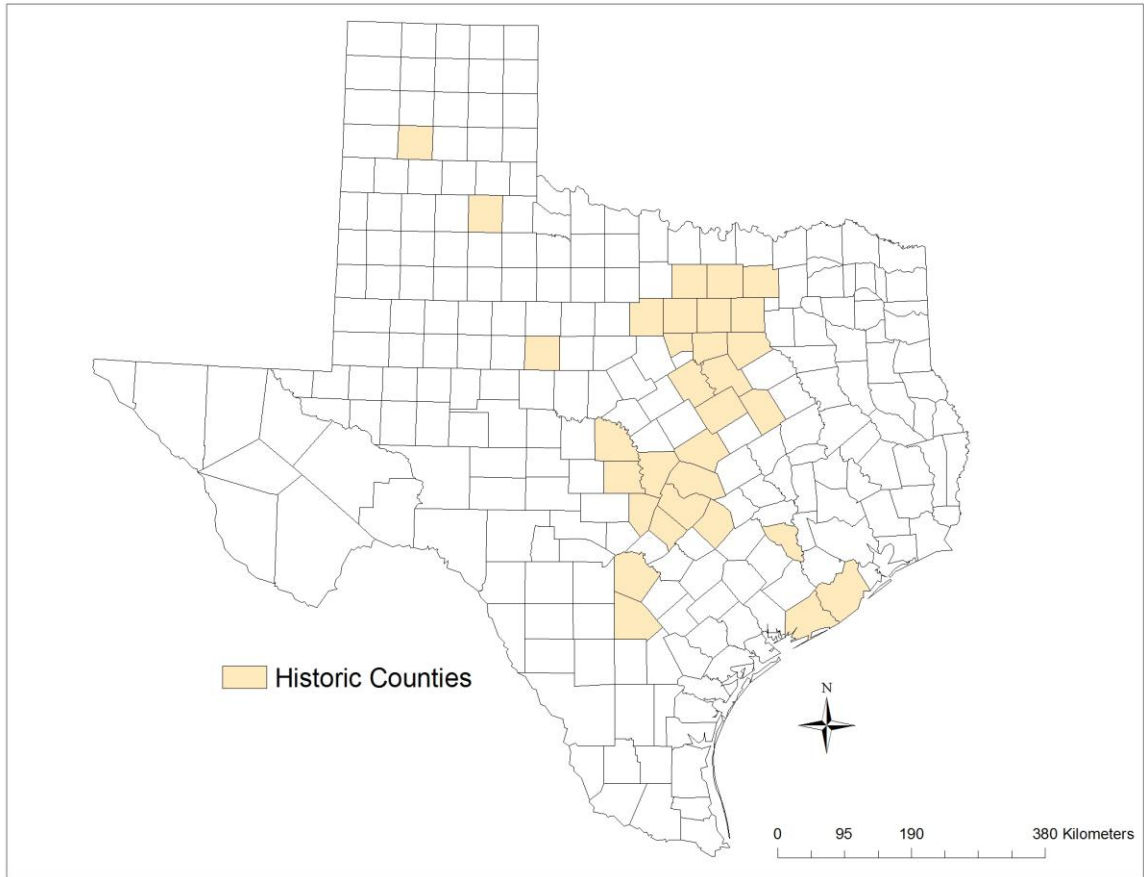


Figure 2. County based map of historic range of *T. s. annectens* in Texas. Historic county range data is compiled from Dixon (2000) and Texas parks and wildlife data.

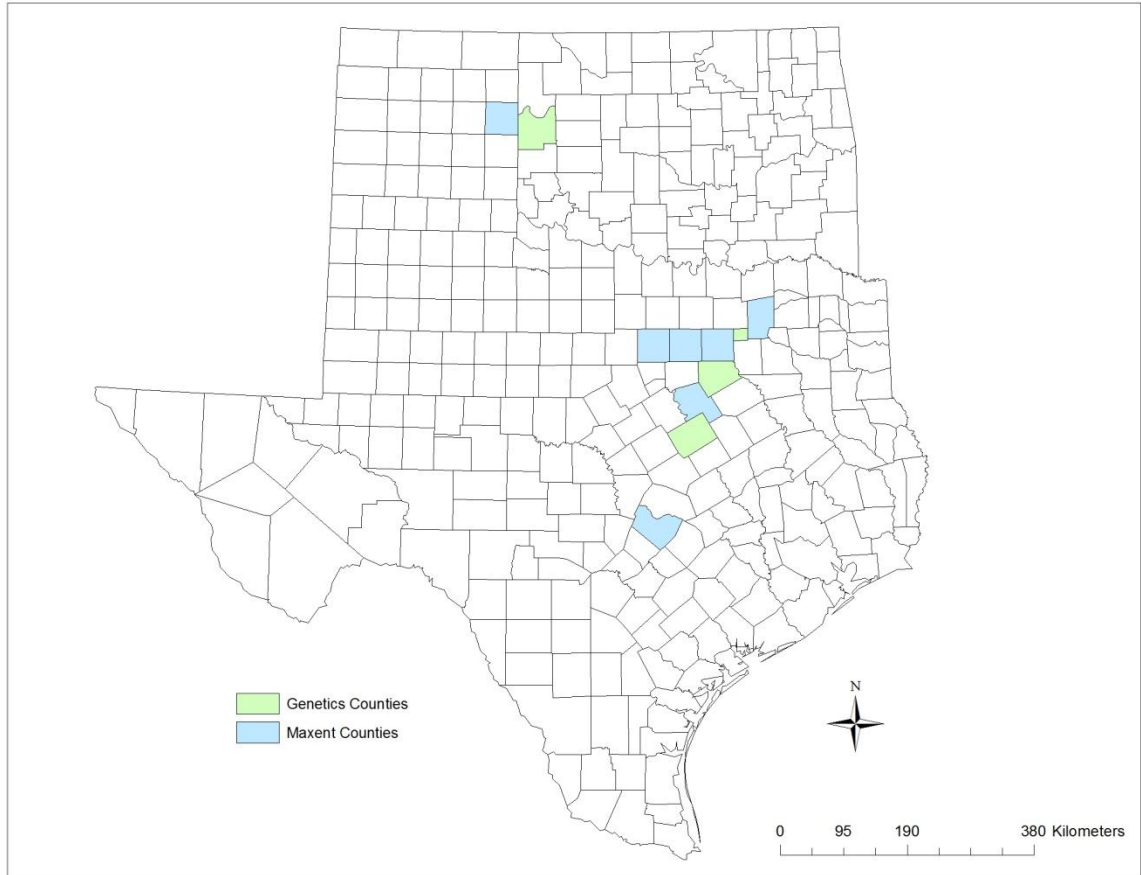


Figure 3. County-based map showing location data of tissue samples of *T. s. annectens* used for genetic analysis and ecological niche modeling.

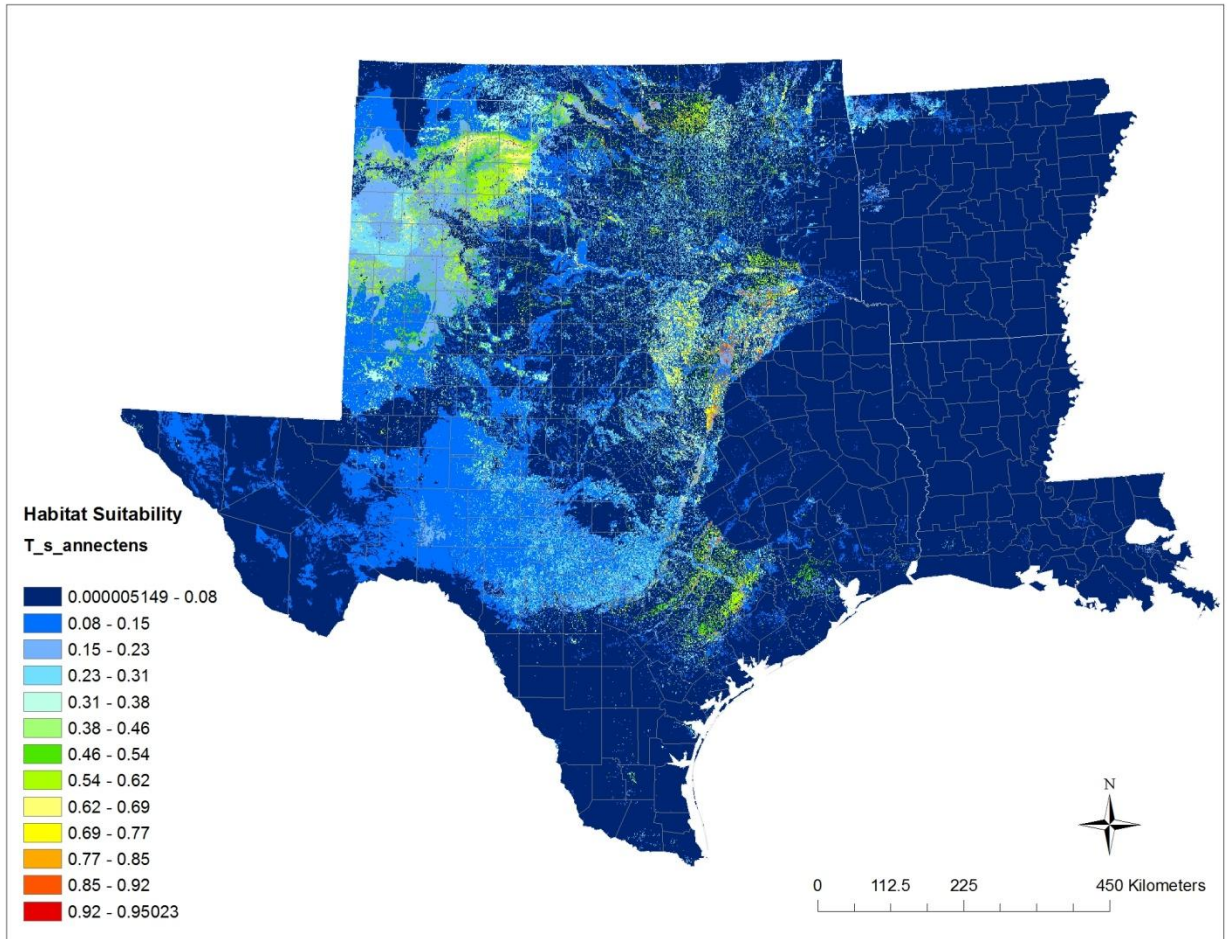


Figure 4. The individual ecological niche model for the Texas gartersnake (*T. s. annectens*). Locality data detailed in Table 2.

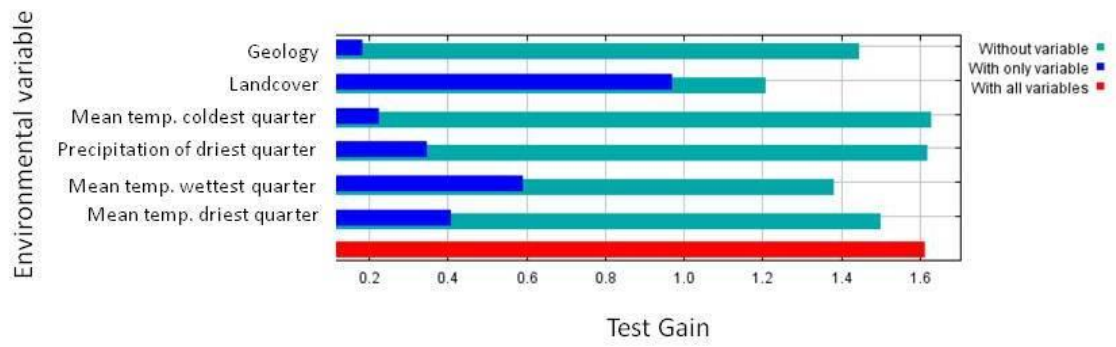


Figure 5. Jackknife of test gain from Maxent for the niche model of the Texas gartersnake (*T. s. annectens*).

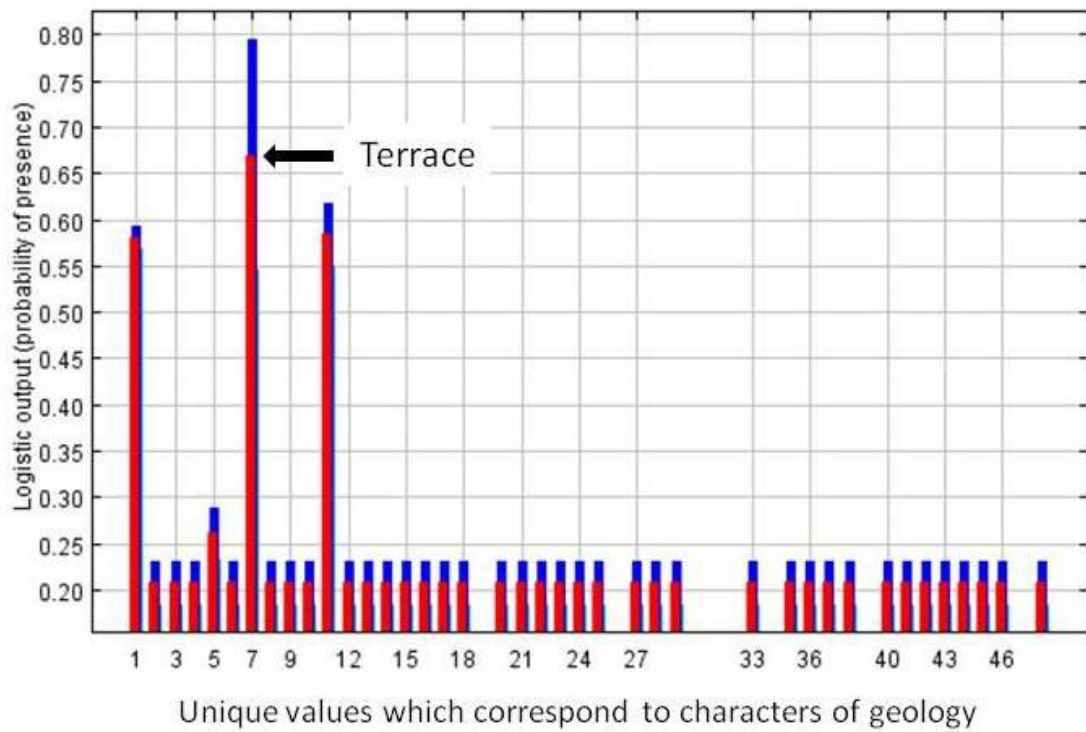


Figure 6. Geology response curve from Maxent showing probability of presence of *T. s. annectens* at a specific geological characteristic. Identification values and corresponding character can be found in Table 8.

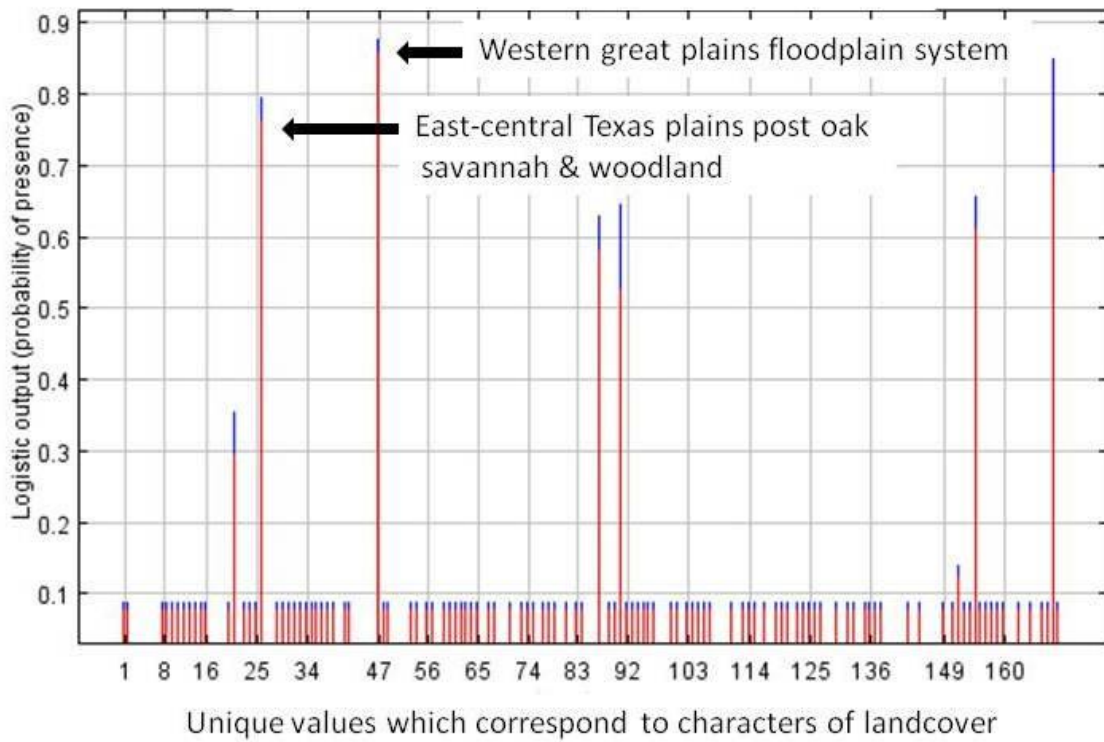


Figure 7. Landcover response curve from Maxent showing probability of presence of *T. s. annexens* at a specific landcover characteristic. Identification values and corresponding character can be found in Table 9.



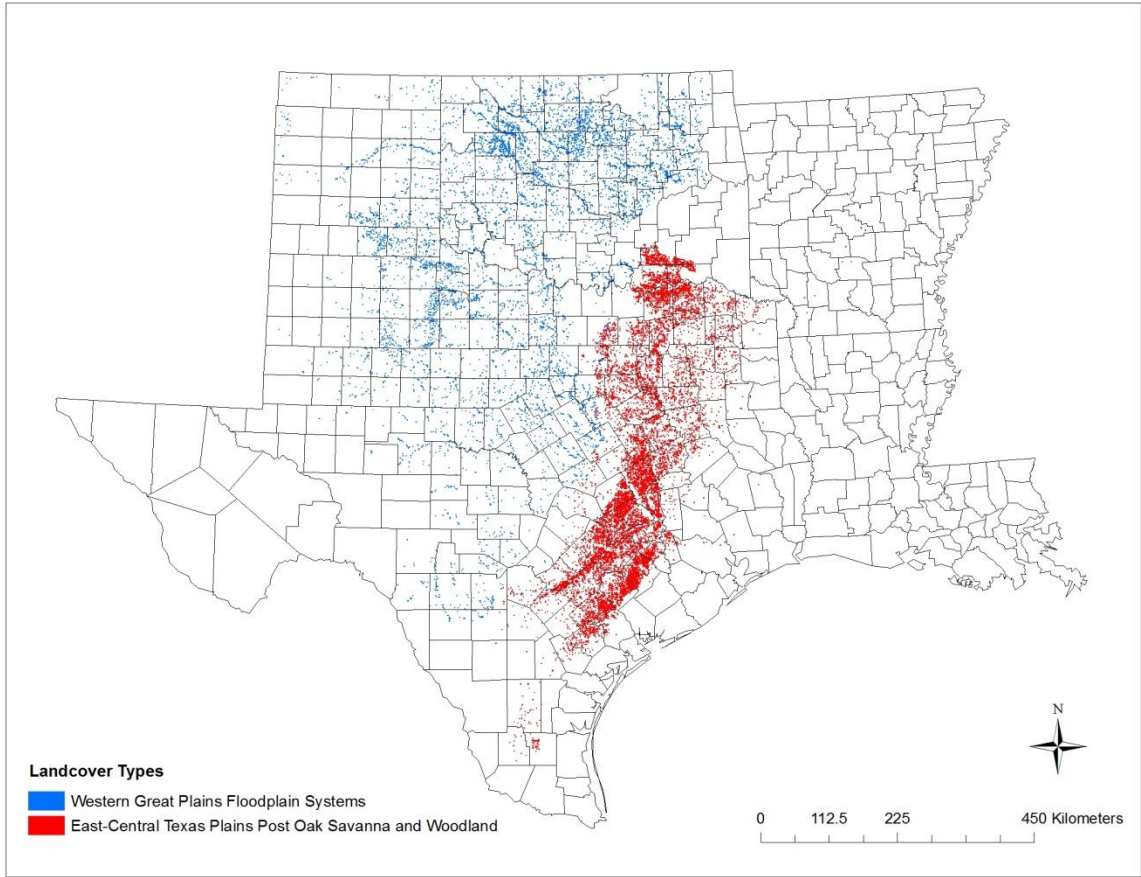


Figure 8. Distribution of the top two most important landcover types for *T. s. annectens*.

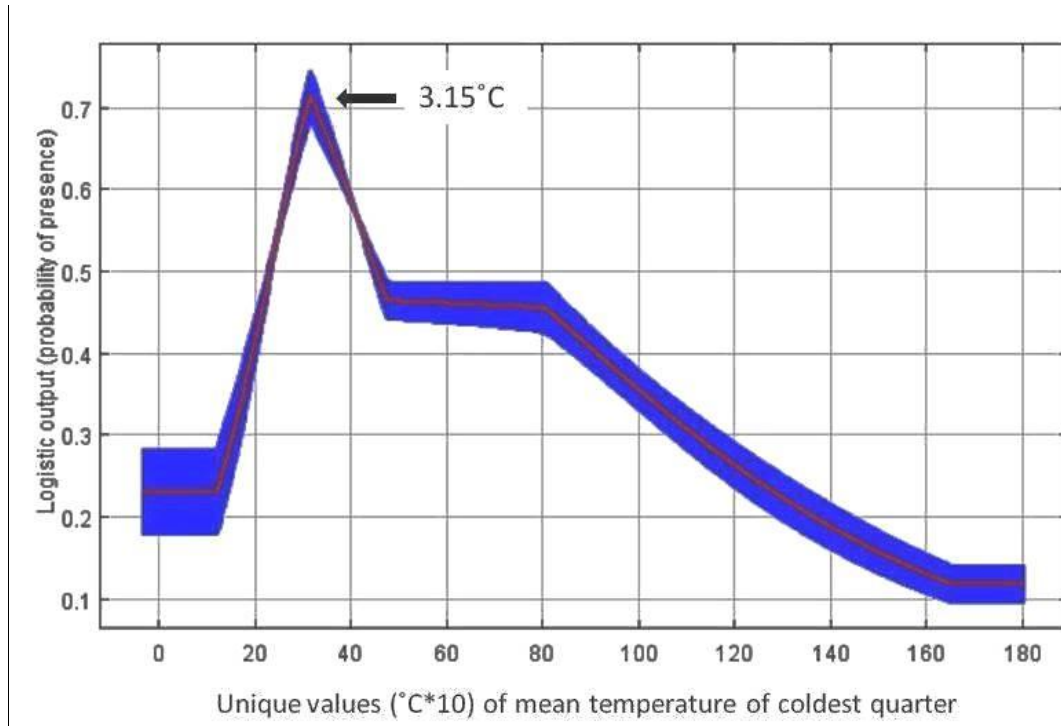


Figure 9. Mean temperature of coldest quarter response curve from Maxent showing probability of presence of *T. s. annectens* at a specific temperature (°C\*10).

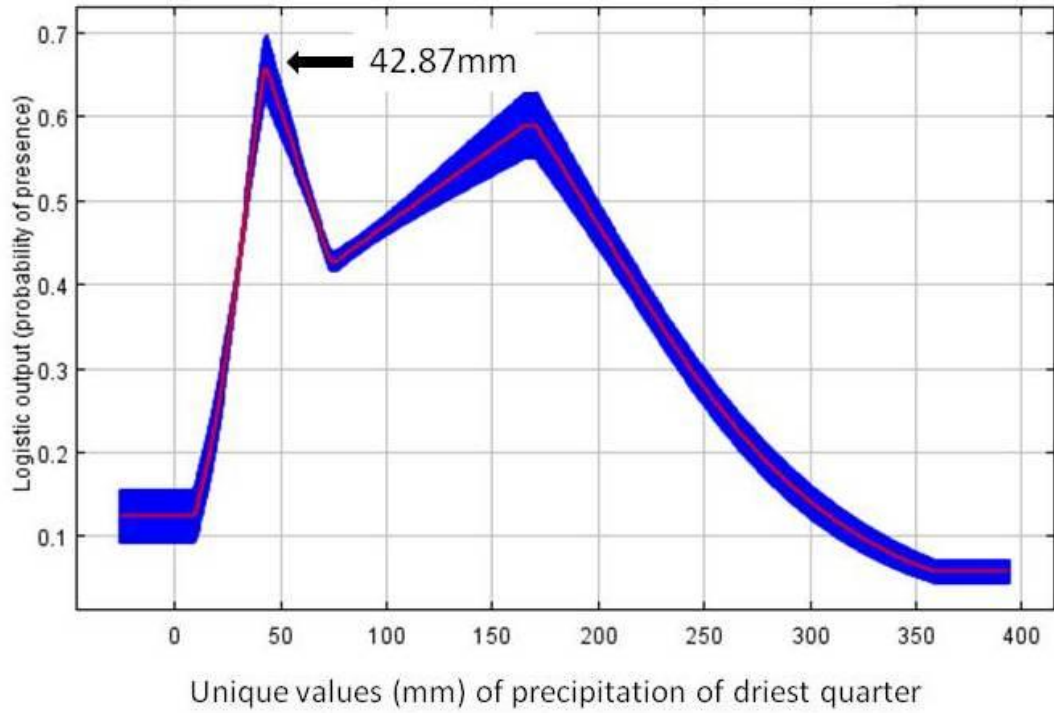


Figure 10. Precipitation of driest quarter response curve from Maxent showing probability of presence of *T. s. annectens* at a specific precipitation amount (mm).

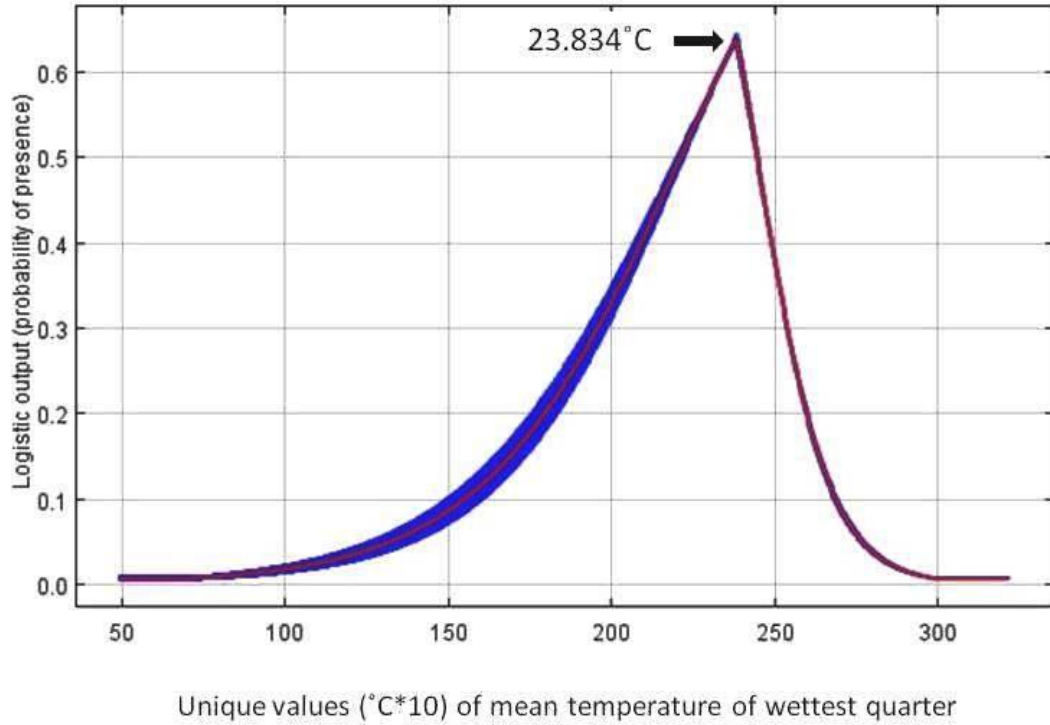


Figure 11. Mean temperature of wettest quarter response curve from Maxent showing probability of presence of *T. s. annectens* at a specific temperature (°C\*10).

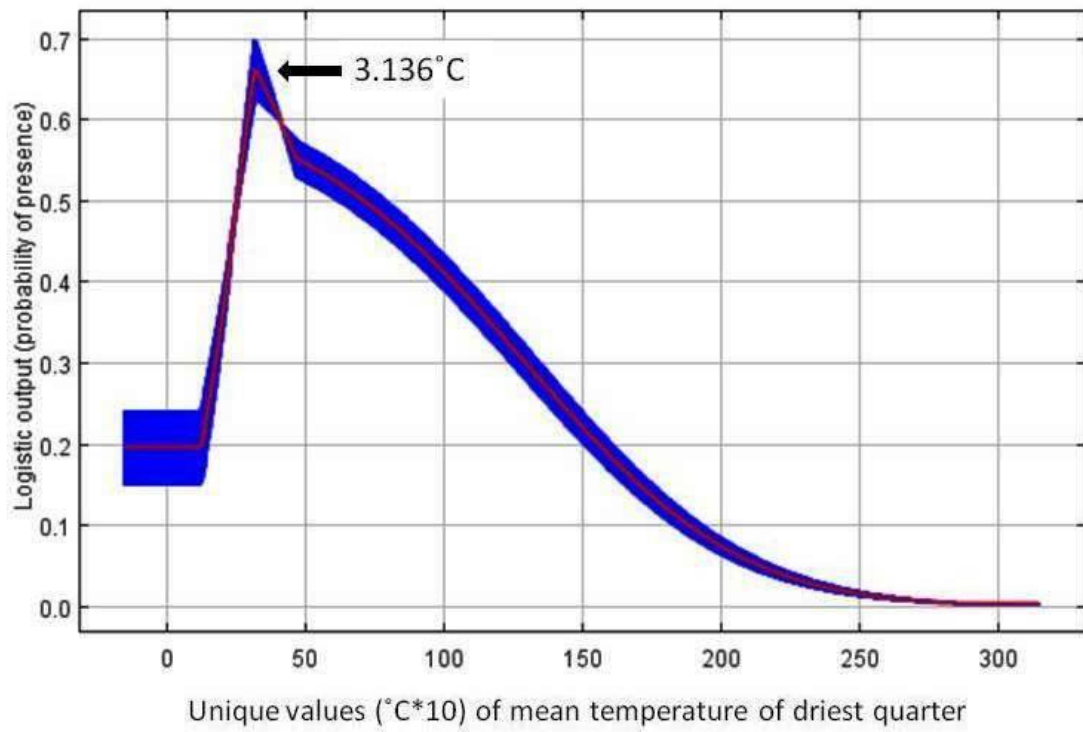


Figure 12. Mean temperature of driest quarter response curve from Maxent showing probability of presence of *T. s. annectens* at a specific temperature (°C\*10).

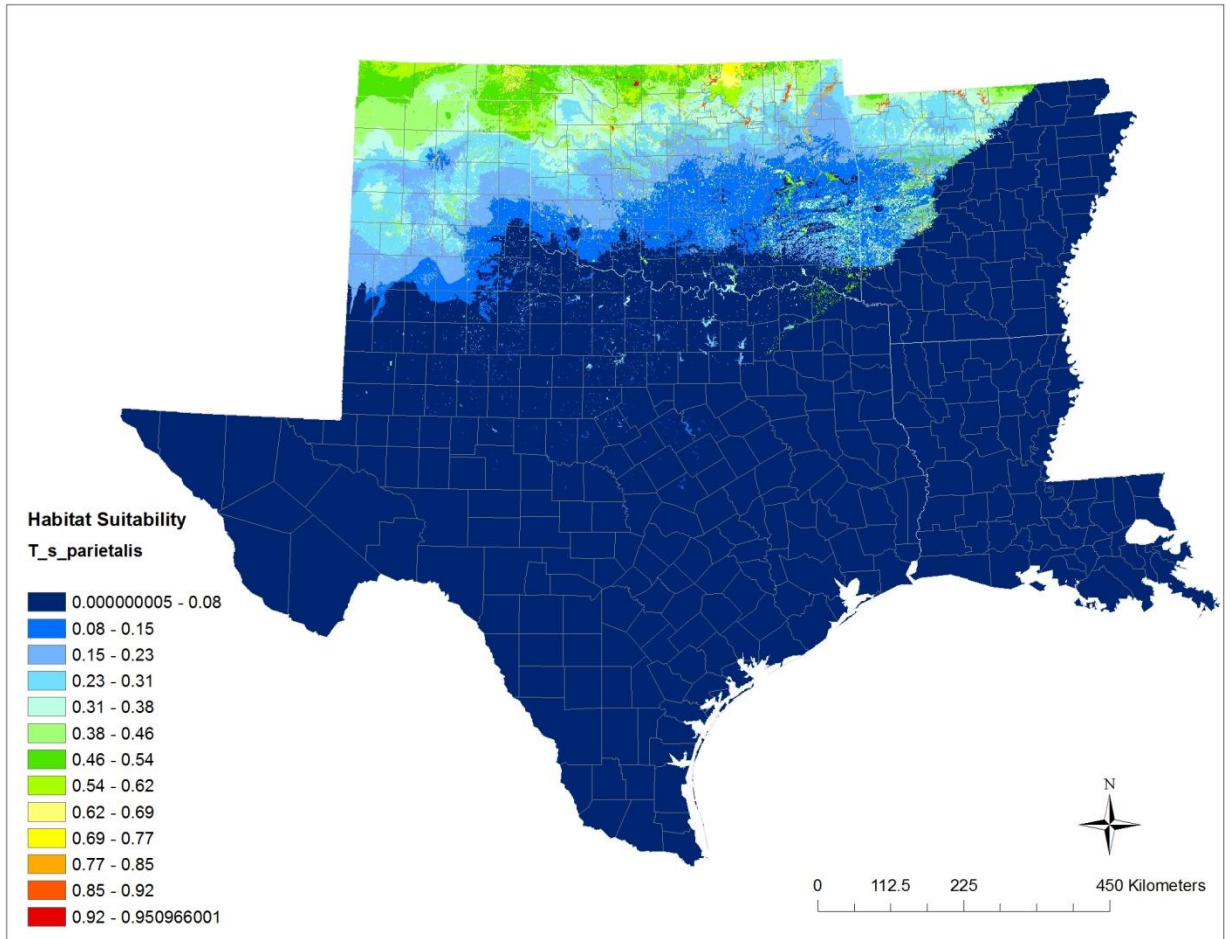


Figure 13. The individual ecological niche model for the red-sided gartersnake (*T. s. parietalis*). Locality data detailed in Table 2.

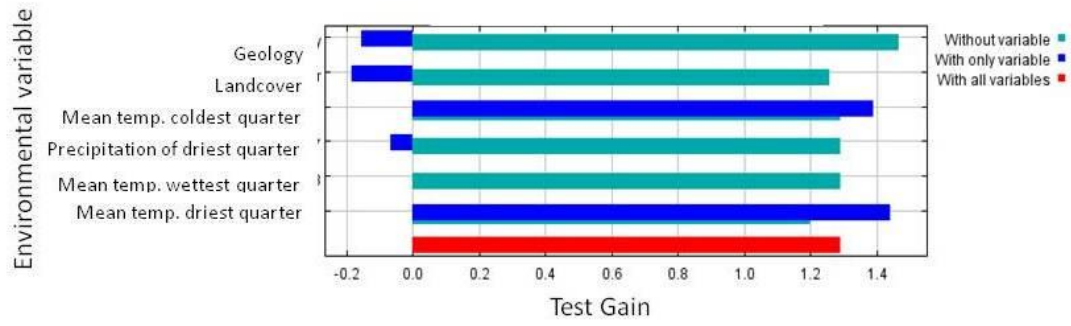


Figure 14. Jackknife of test gain from Maxent for the niche model of the red-sided gartersnake (*T. s. parietalis*).

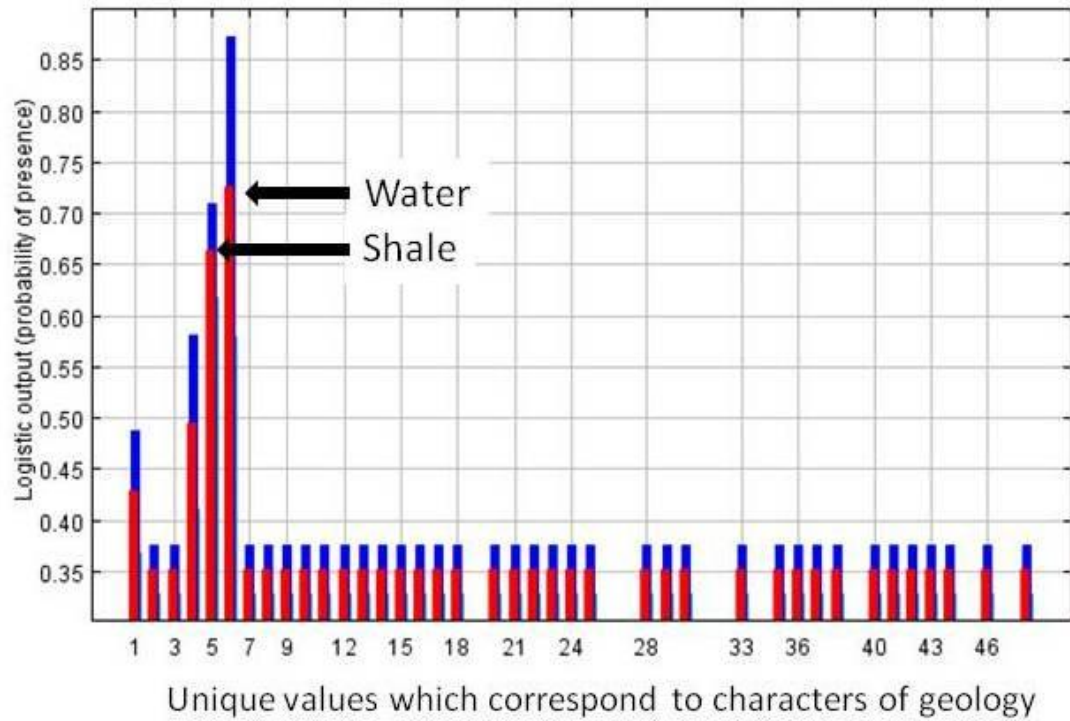


Figure 15. Geology response curve from Maxent showing probability of presence of *T. s. parietalis* at a specific geological characteristic. Identification values and corresponding character can be found in Table 8.



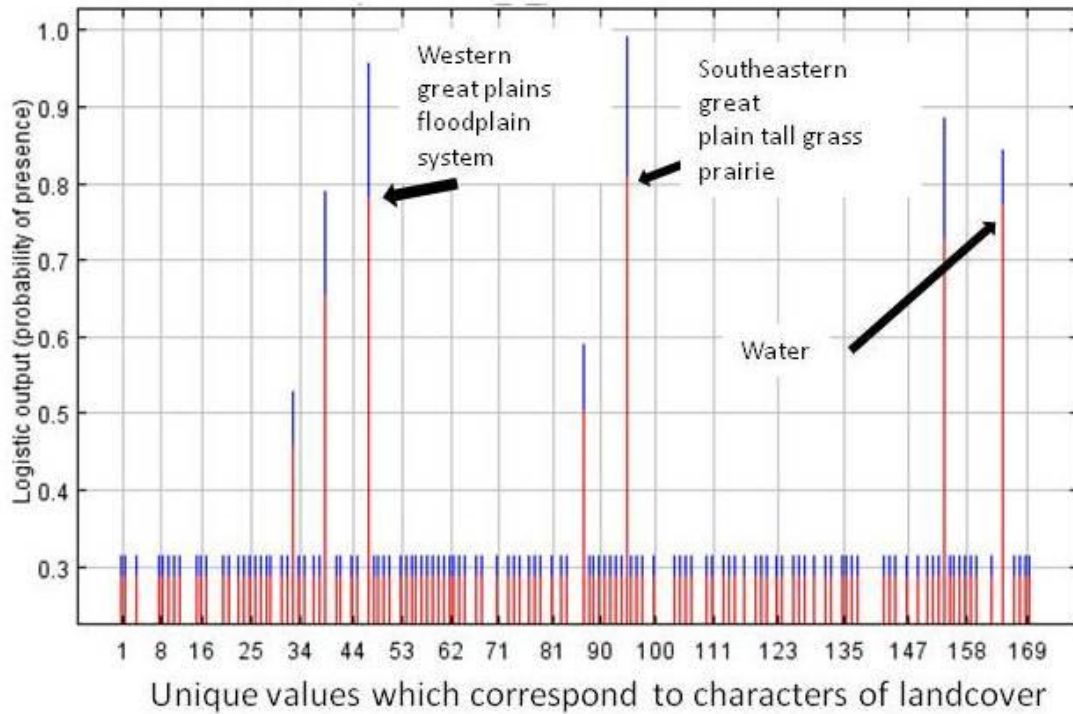


Figure 16. Landcover response curve from Maxent showing probability of presence of *T. s. parietalis* at a specific landcover characteristic. Identification values and corresponding character can be found in Table 9.

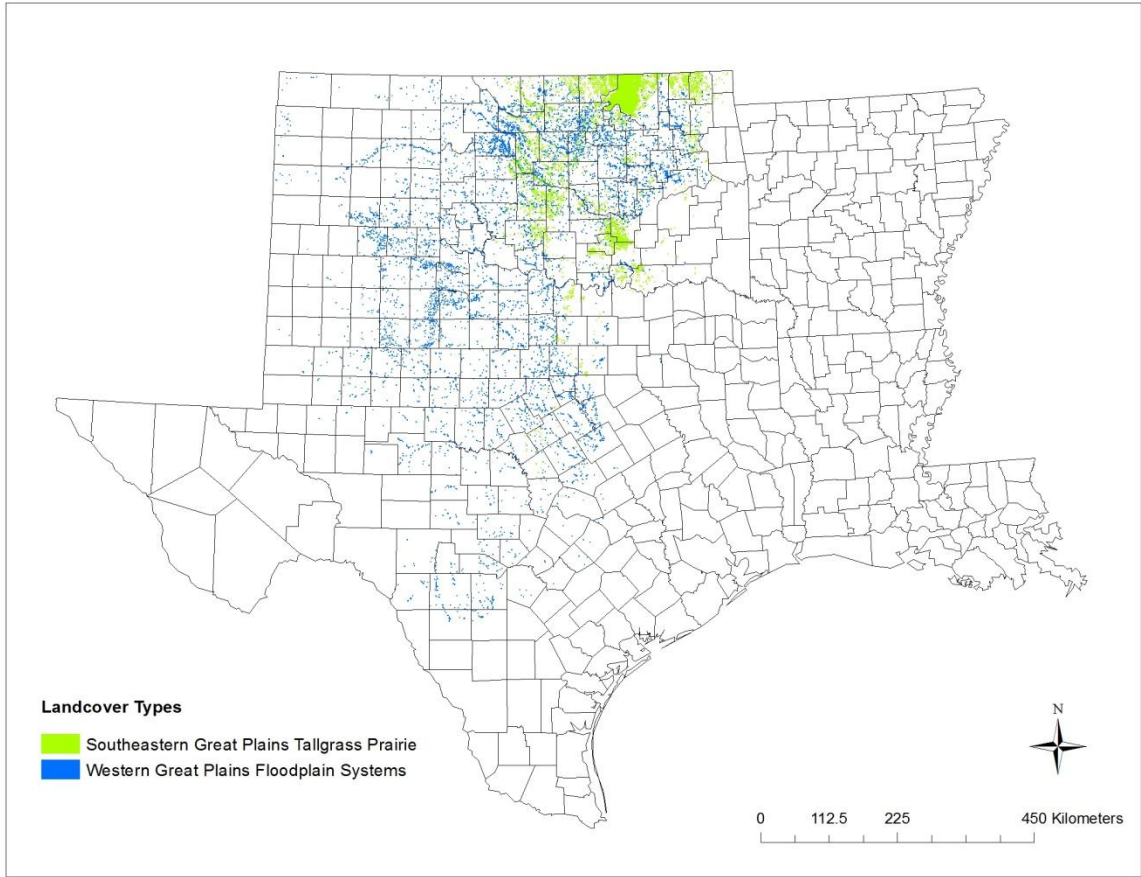


Figure 17. Distribution of the top two most important landcover types for *T. s. parietalis*.

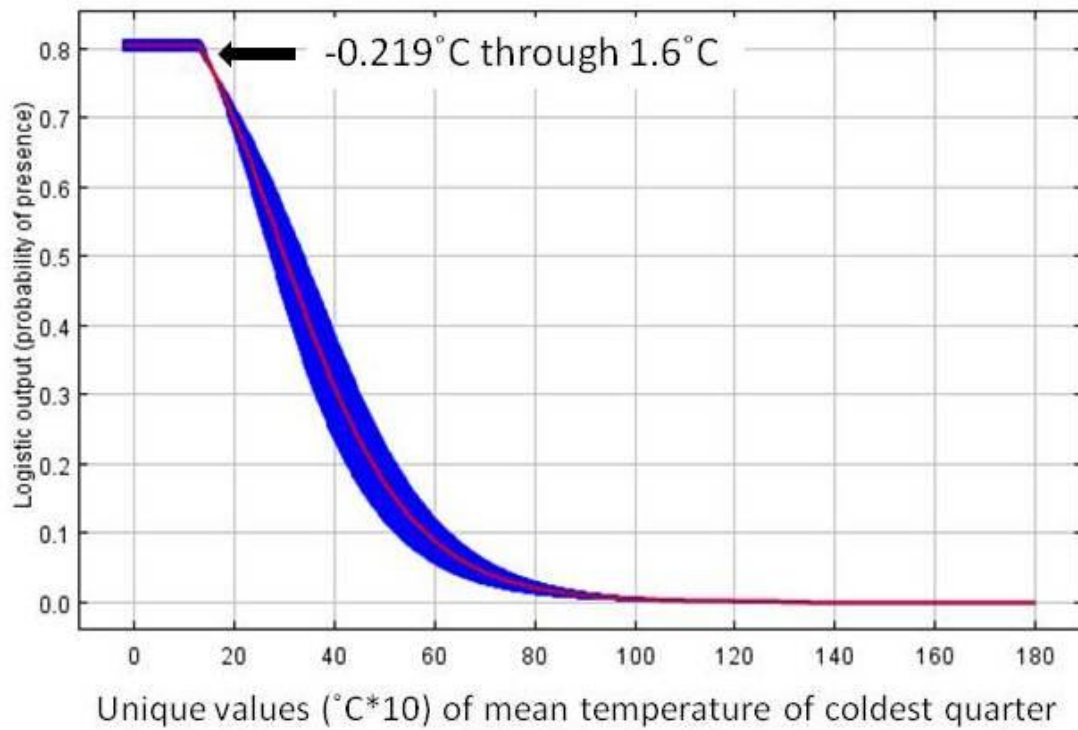


Figure 18. Mean temperature of coldest quarter response curve from Maxent showing probability of presence of *T. s. parietalis* at a specific temperature (°C\*10).

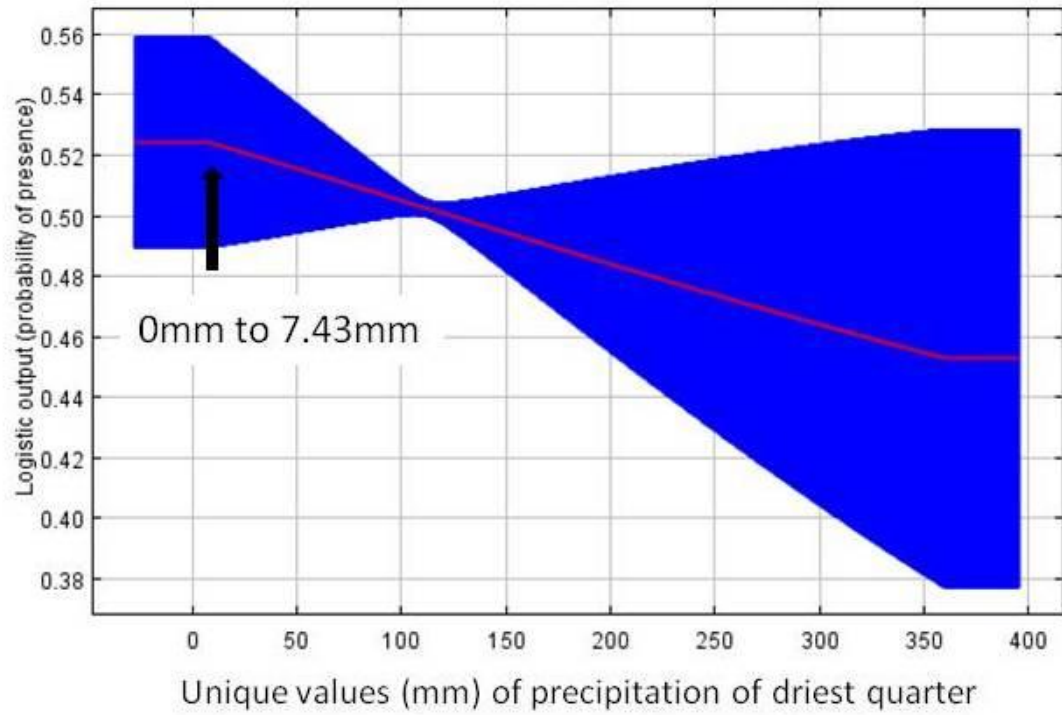


Figure 19. Precipitation of driest quarter response curve from Maxent showing probability of presence of *T. s. parietalis* at a specific precipitation amount (mm).

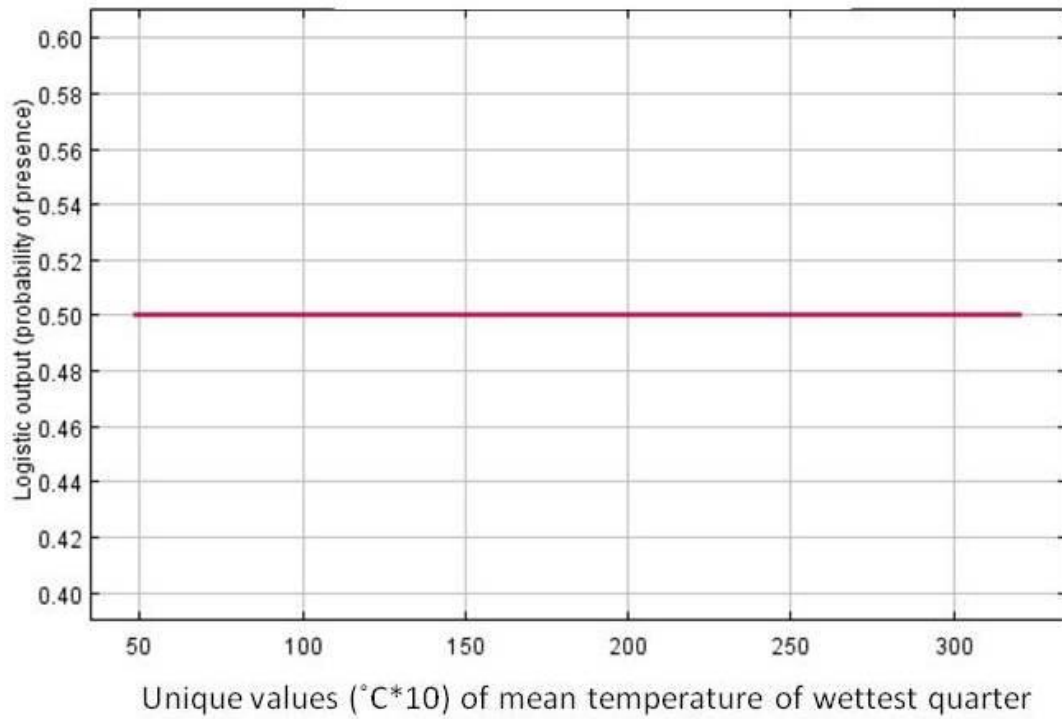


Figure 20. Mean temperature of wettest quarter response curve from Maxent showing probability of presence of *T. s. parietalis* at a specific temperature (°C\*10).

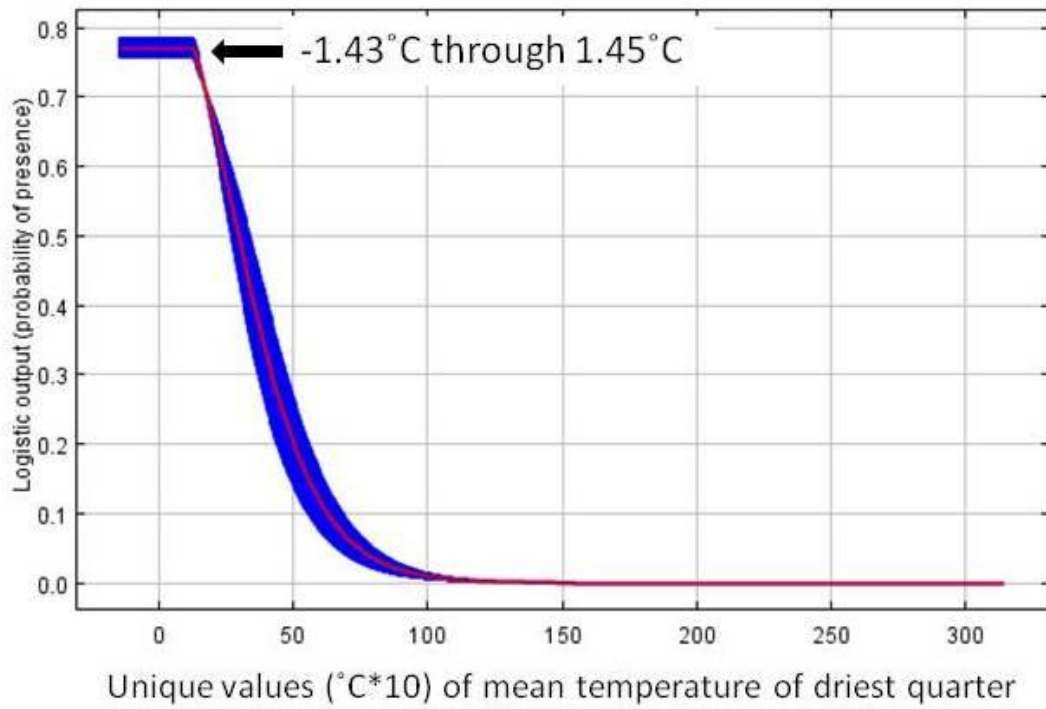


Figure 21. Mean temperature of driest quarter response curve from Maxent showing probability of presence of *T. s. parietalis* at a specific temperature (°C\*10).

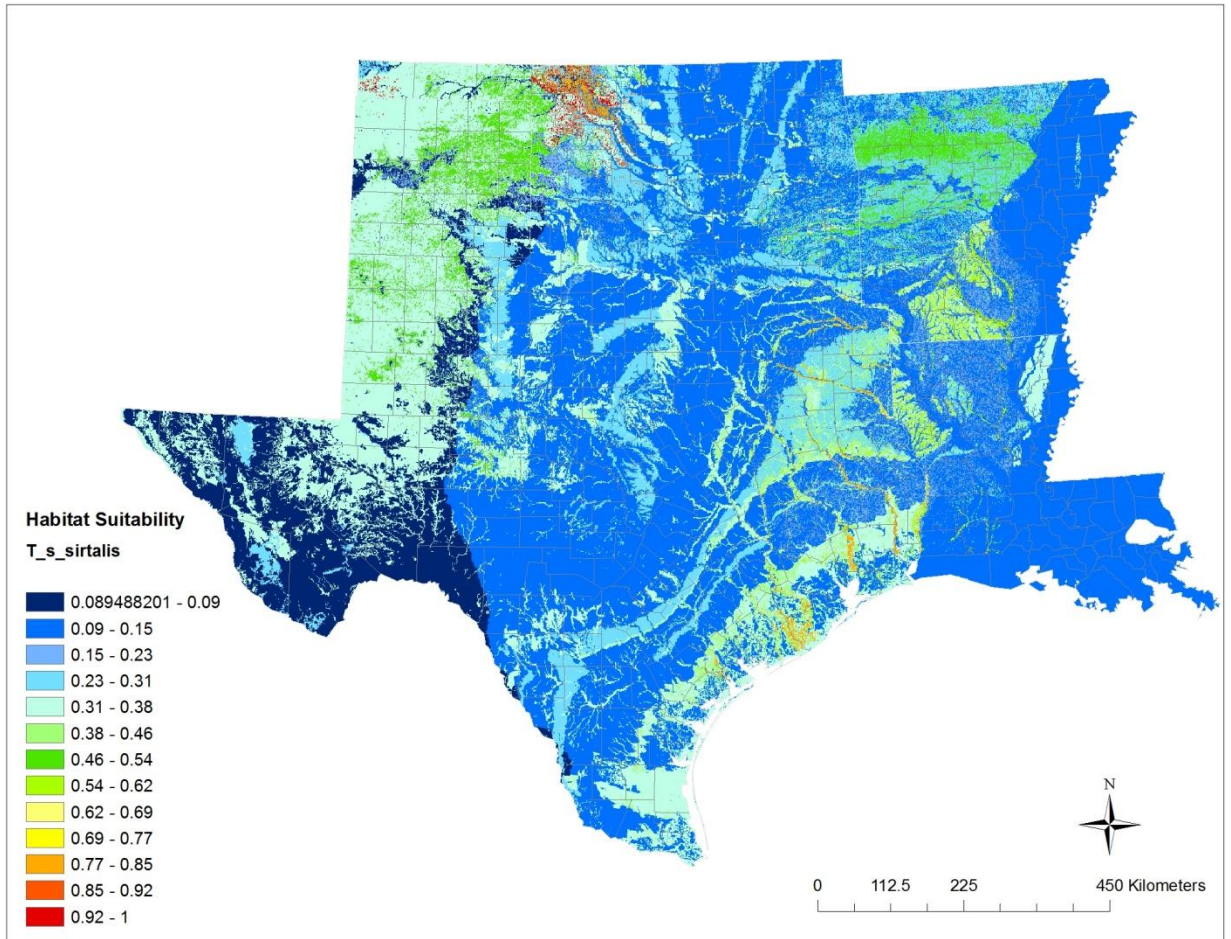


Figure 22. The individual ecological niche model for the eastern gartersnake (*T. s. sirtalis*). Locality data detailed in Table 2.

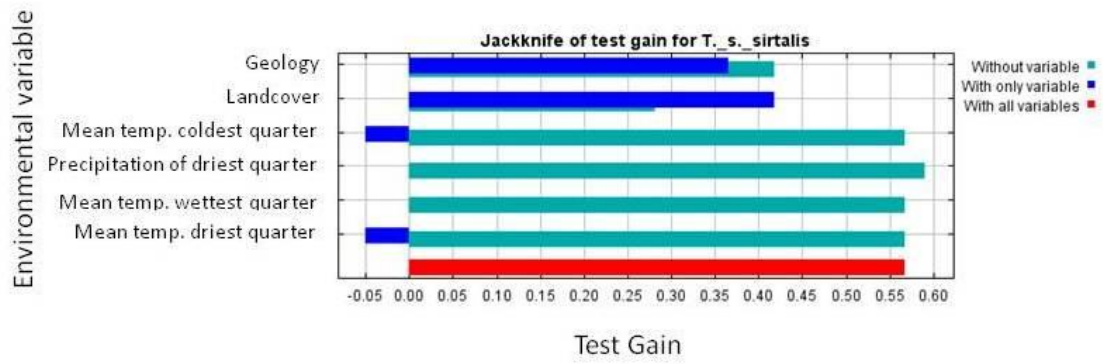


Figure 23. Jackknife of test gain from Maxent for the niche model for the eastern gartersnake (*T. s. sirtalis*).



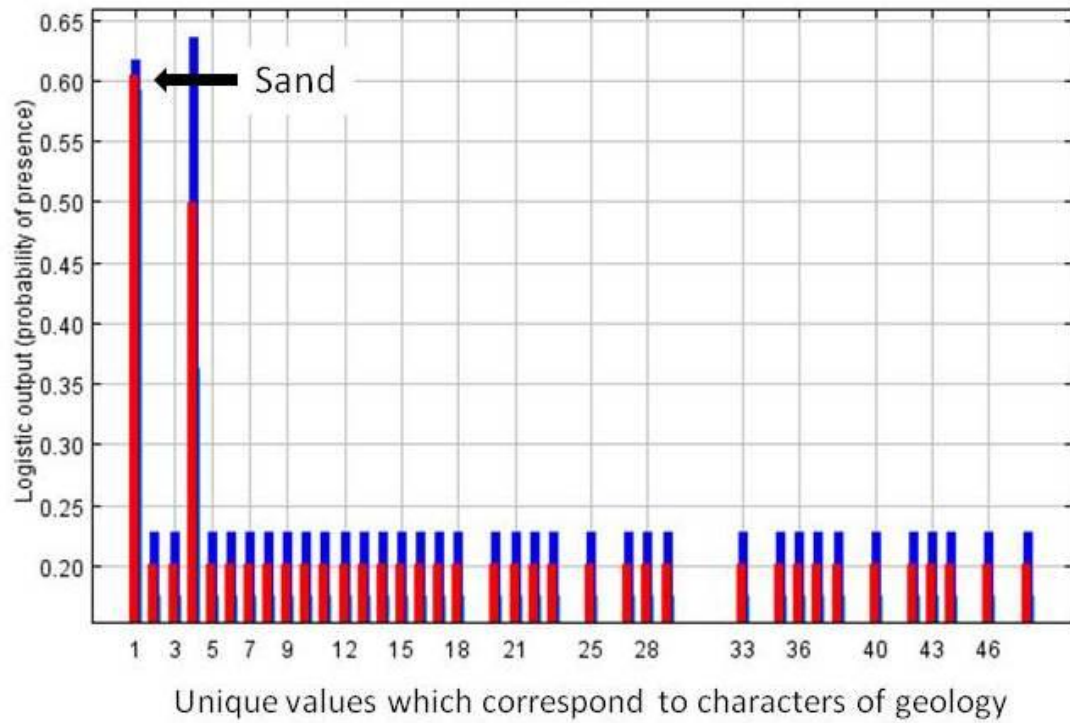


Figure 24. Geology response curve from Maxent showing probability of presence of *T. s. sirtalis* at a specific geological characteristic. Identification values and corresponding character can be found in Table 8.

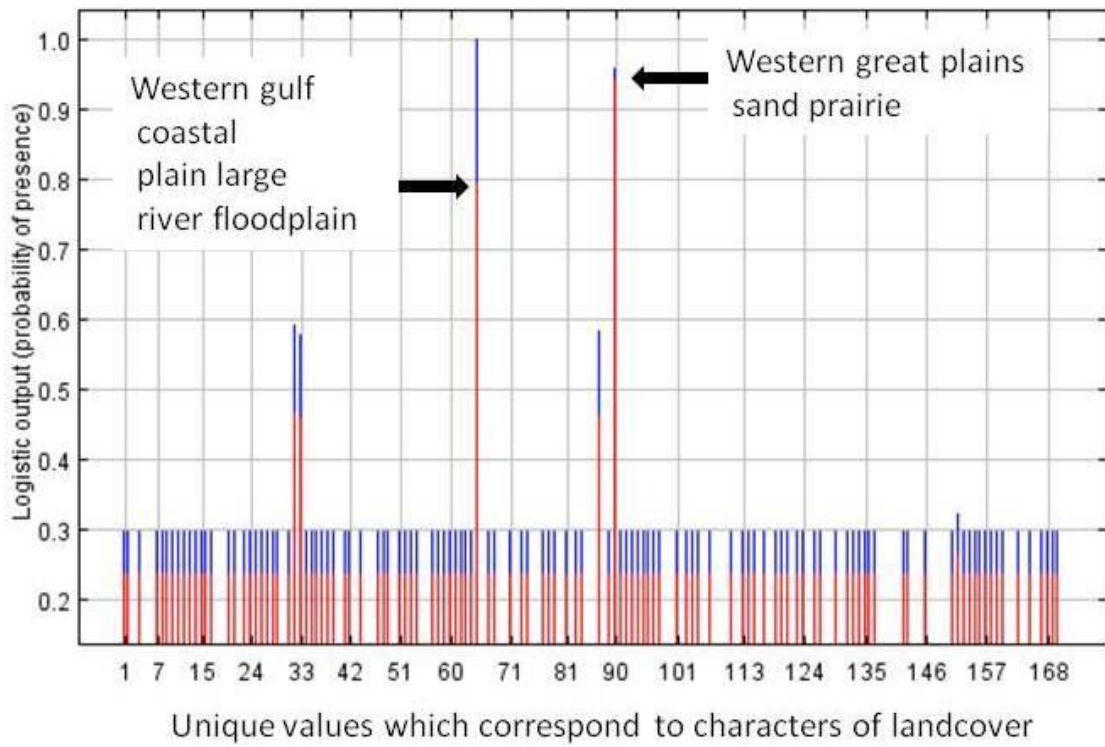


Figure 25. Landcover response curve from Maxent showing probability of presence of *T. s. sirtalis* at a specific landcover characteristic. Identification values and corresponding character can be found in Table 9.

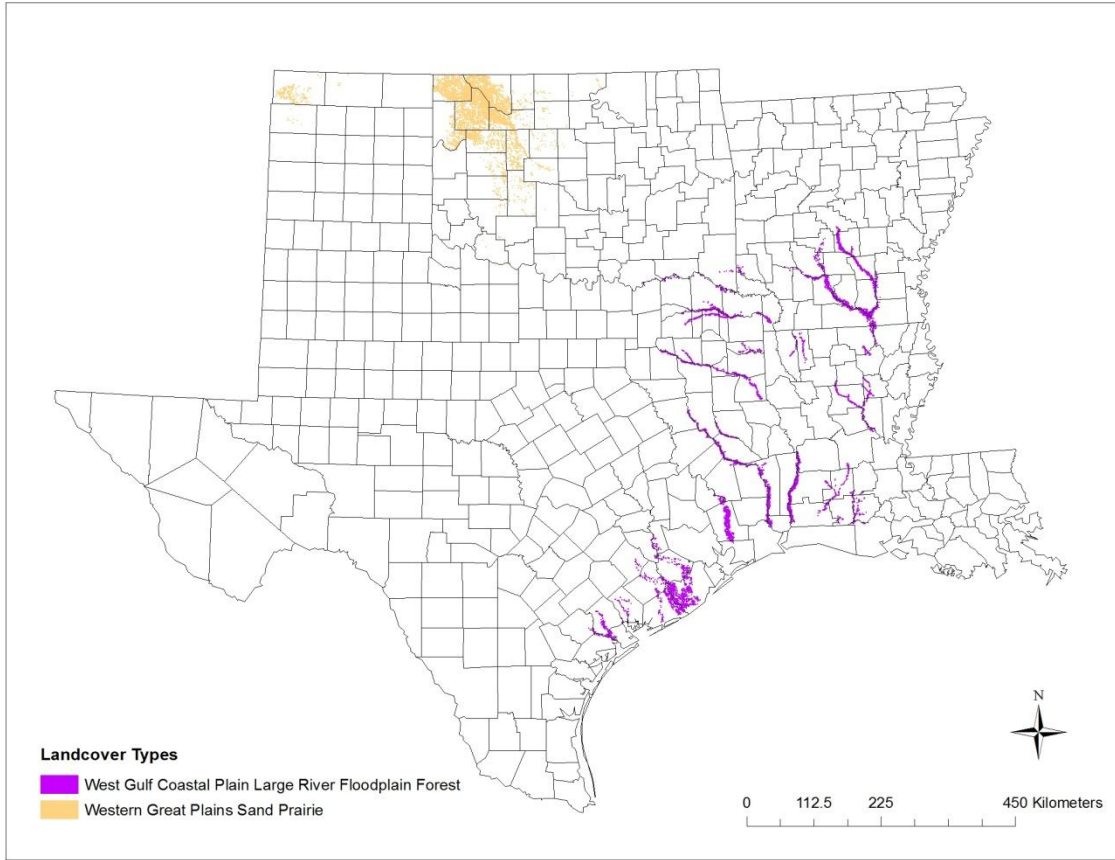


Figure 26. Distribution of the top two most important landcover types for *T. s. sirtalis*.

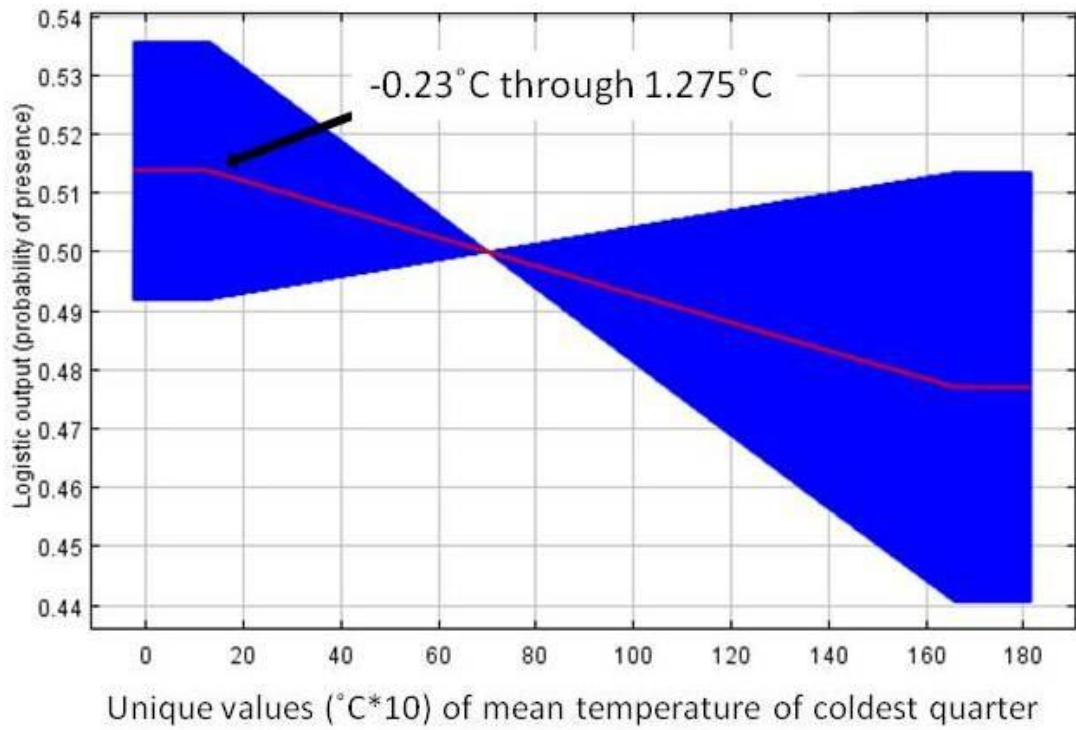


Figure 27. Mean temperature of coldest quarter response curve from Maxent showing probability of presence of *T. s. sirtalis* at a specific temperature (°C\*10).

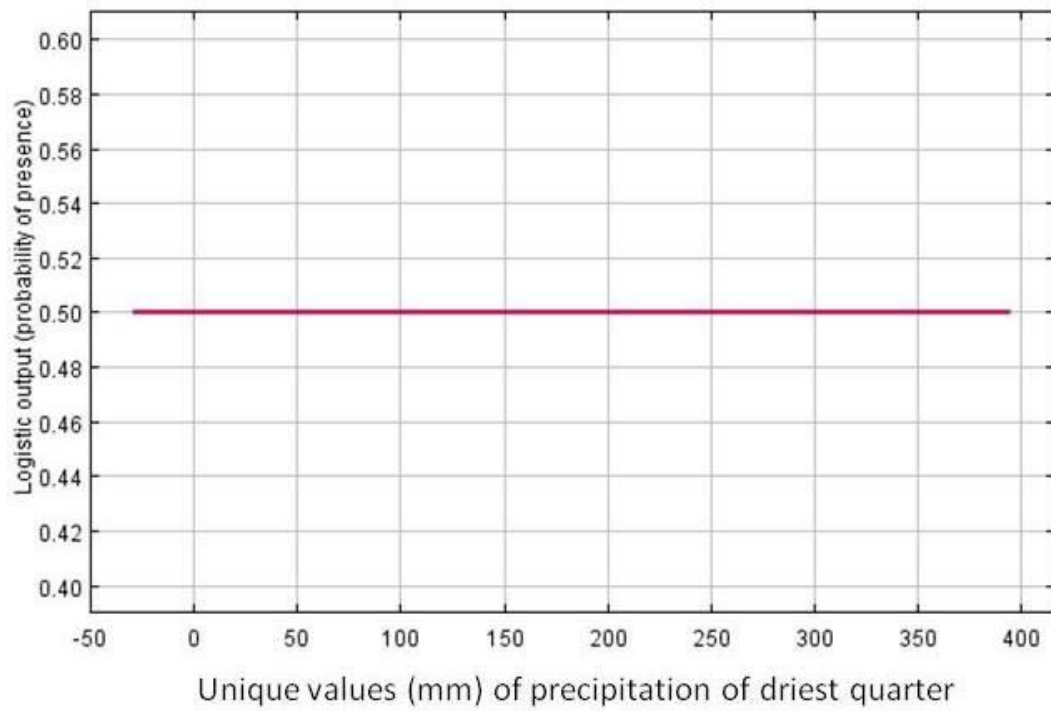


Figure 28. Precipitation of driest quarter response curve from Maxent showing probability of presence of *T. s. sirtalis* at a specific precipitation amount (mm).

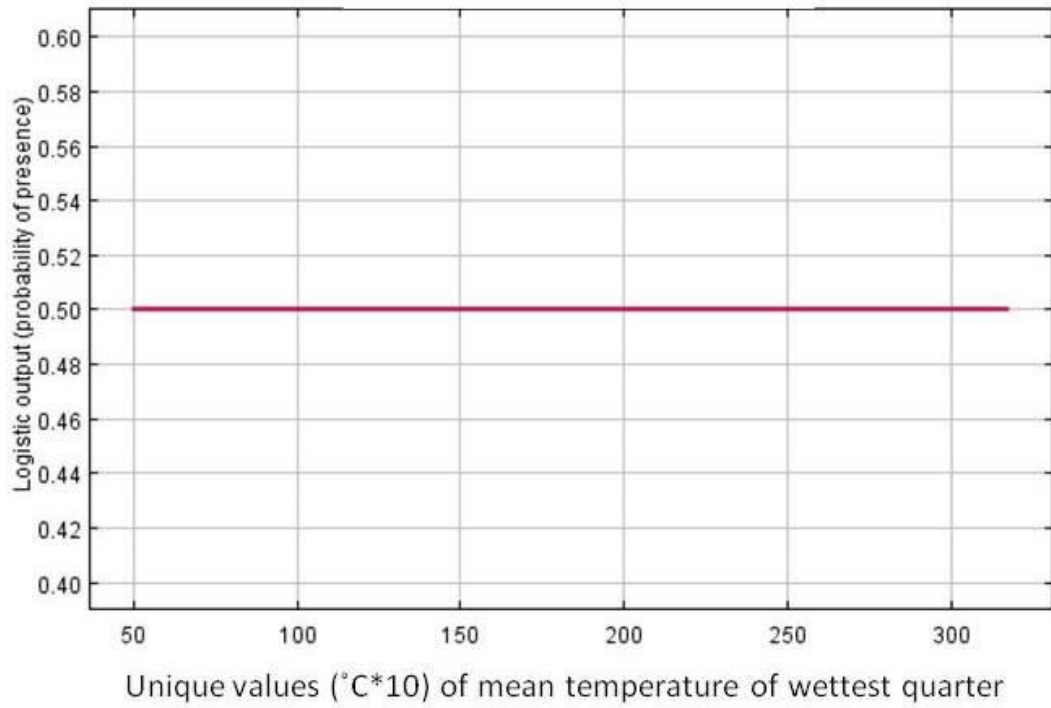


Figure 29. Mean temperature of wettest quarter response curve from Maxent showing probability of presence of *T. s. sirtalis* at a specific temperature (°C\*10).

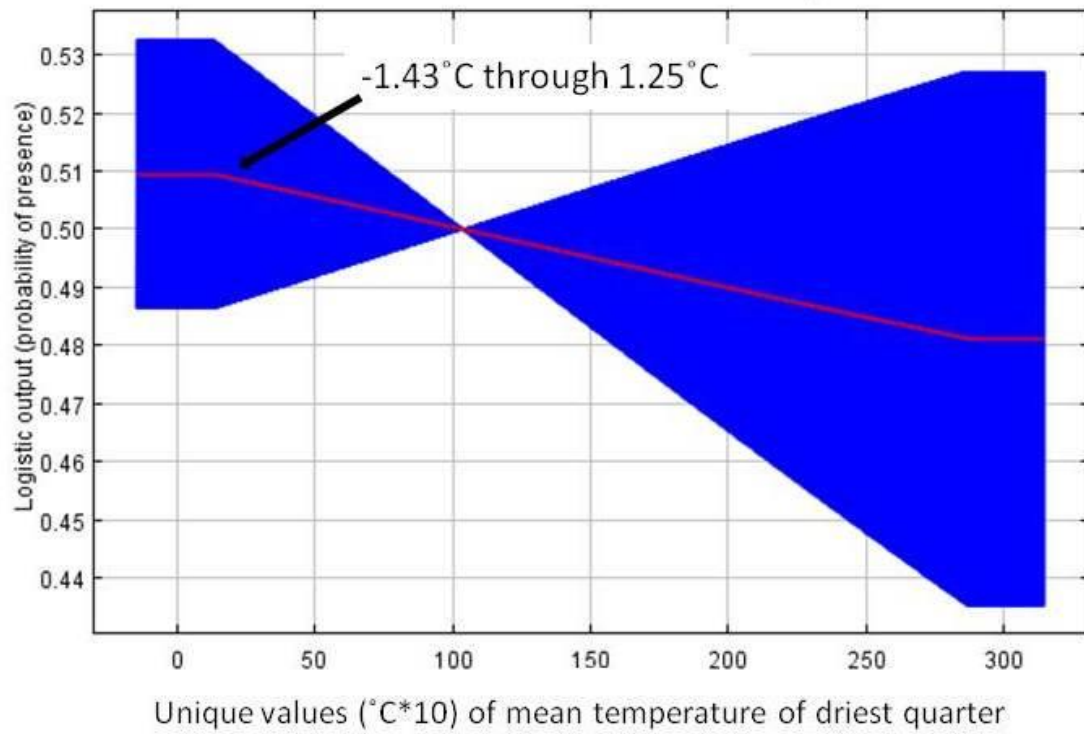


Figure 30. Mean temperature of driest quarter response curve from Maxent showing probability of presence of *T. s. sirtalis* at a specific temperature (°C\*10).

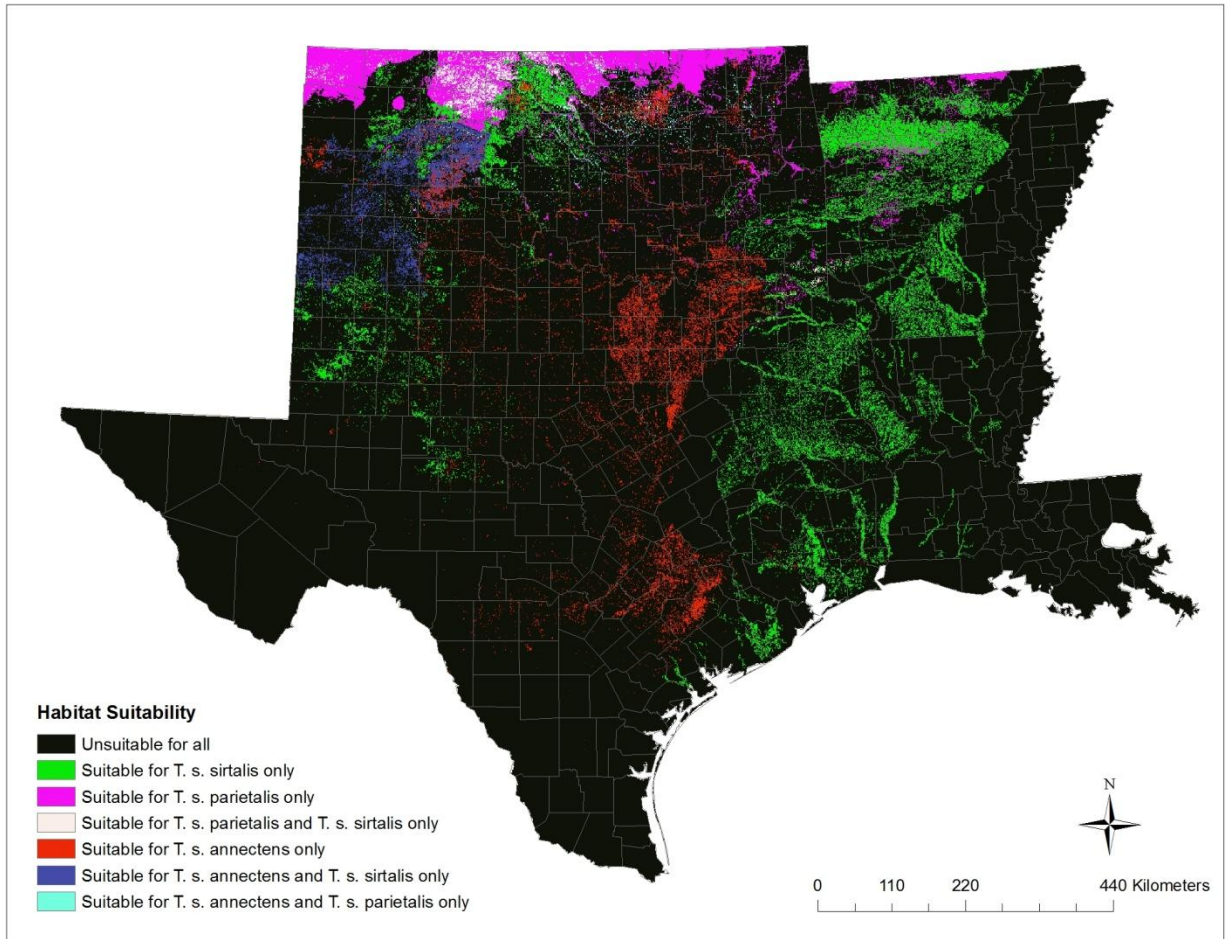


Figure 31. Comparative niche model showing areas of overlap and isolation between the eastern gartersnake (*Thamnophis s. sirtalis*), the red-sided gartersnake (*T. s. parietalis*), and the Texas gartersnake (*T. s. annectens*). Based off binary threshold maps for each subspecies produced from average maxent habitat suitability map outputs.



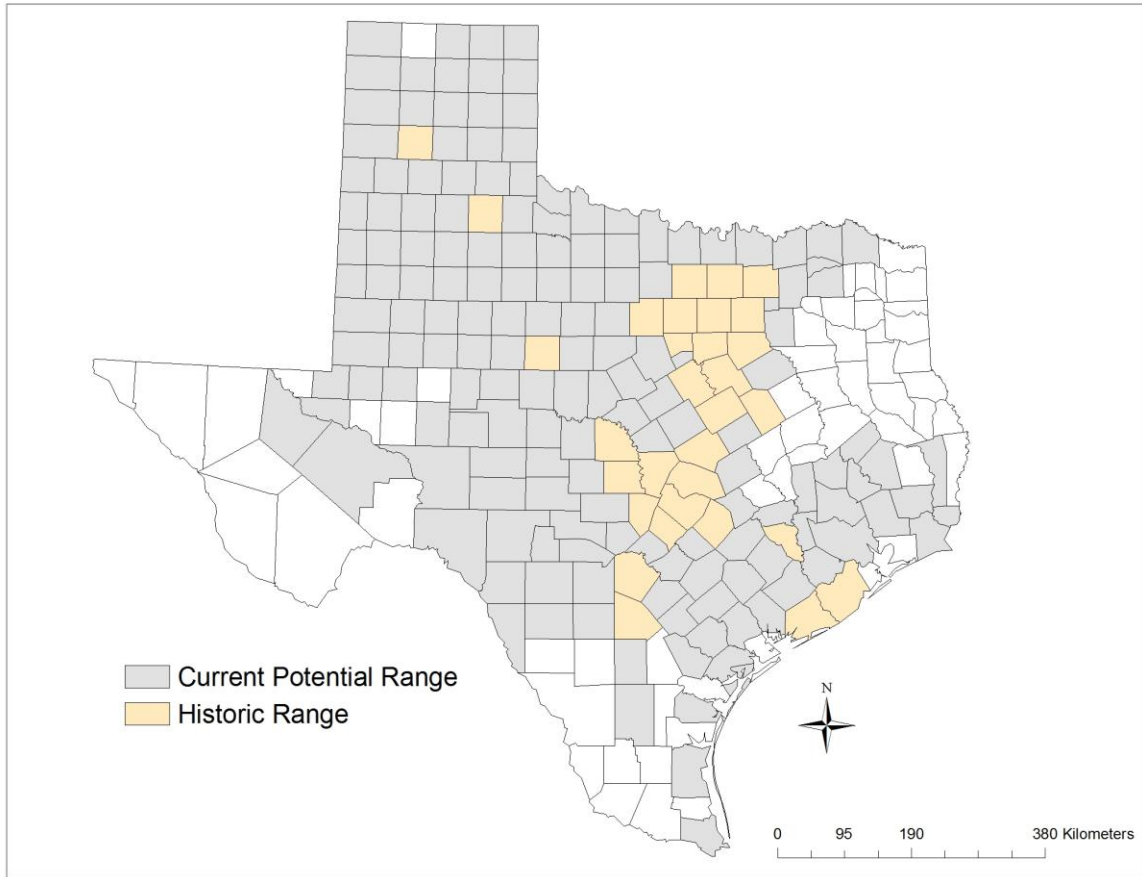


Figure 32. County-based map of the historic range and the current potential range for *T. s. annectens*. Historic range data is compiled from Dixon (2000) and Texas parks and wildlife data. Current potential range data is a representation of the binary threshold map produced from average Maxent habitat suitability map output.

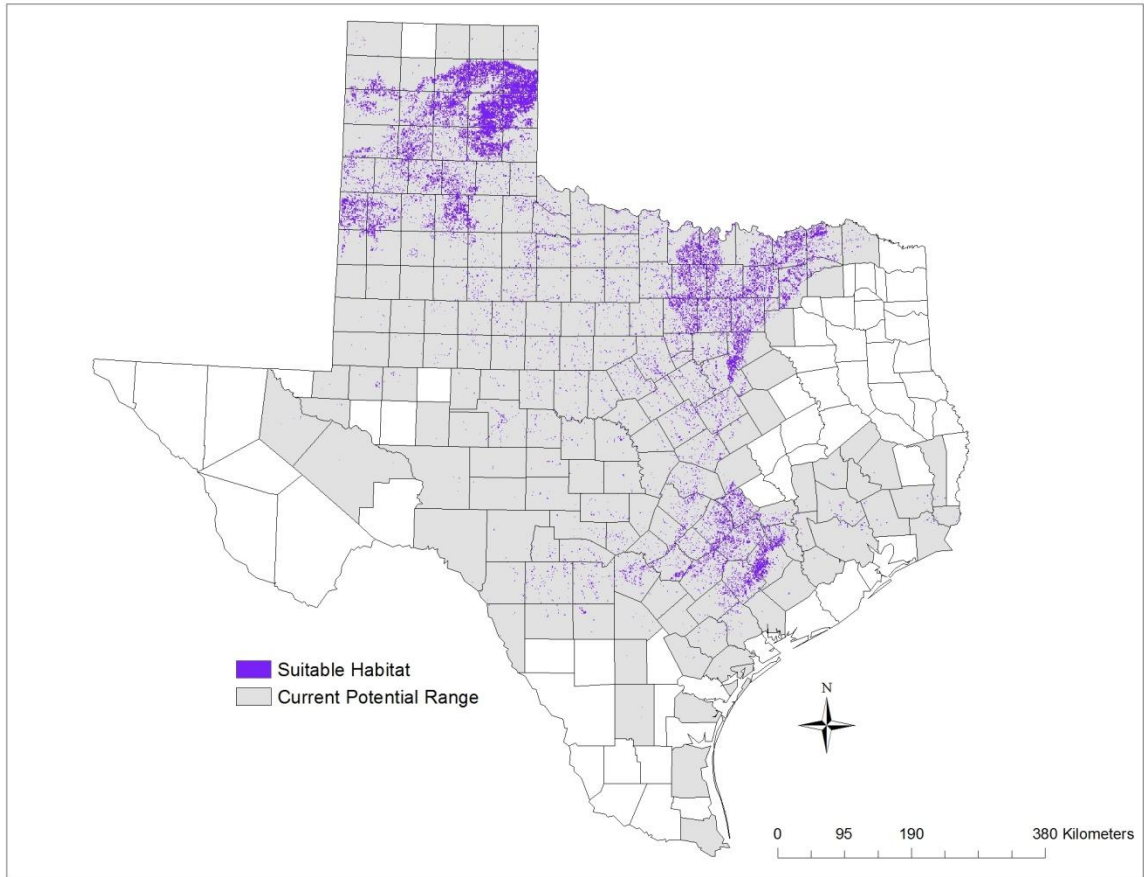


Figure 33. Binary threshold habitat suitability map, based off average Maxent habitat suitability map, overlaid onto current potential county range map for *T. s. annectens*.

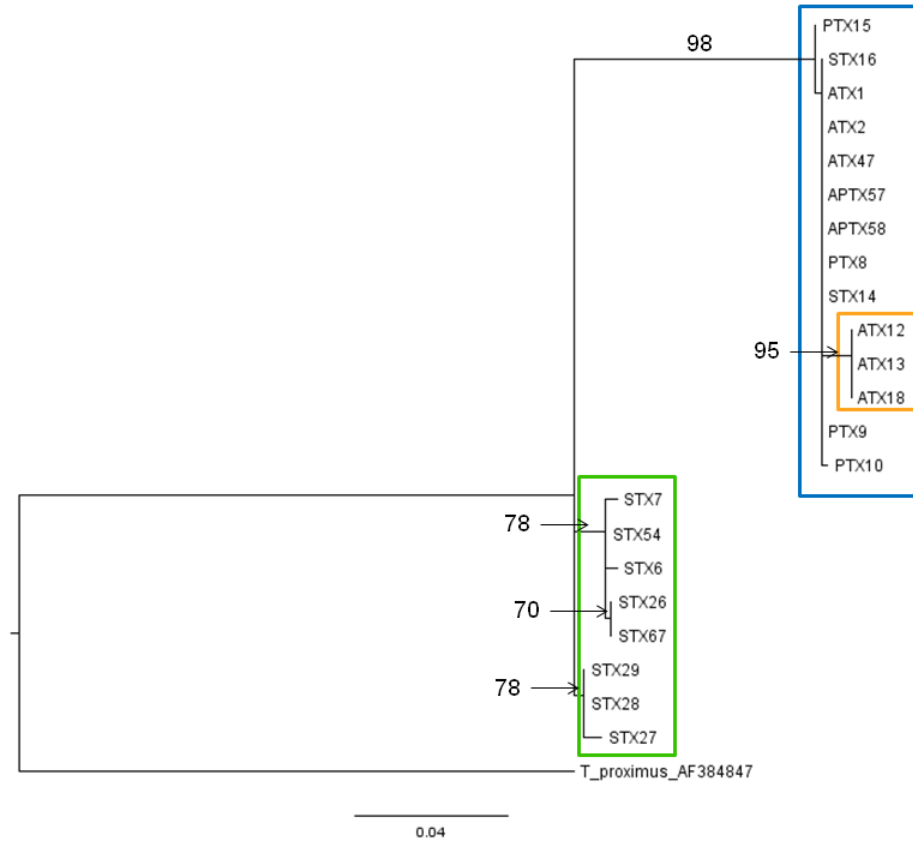


Figure 34. Rooted maximum likelihood phylogeny of the mtDNA gene ND2 for the eastern gartersnake (*Thamnophis s. sirtalis*), the red-sided gartersnake (*T. s. parietalis*), and the Texas gartersnake (*T. s. annectens*). ATX represent *T. s. annectens*. APTX represent individuals collected where the ranges of *T. s. annectens* and *T. s. parietalis* met. PTX represent *T. s. parietalis*. STX represent *T. s. sirtalis*. Numbers to the left indicate non-parametric bootstrap values (>50%) for those recovered in maximum likelihood analysis. Additional data for individuals used in this analysis can be found in Table 4. Colored circles correspond with locality data (Figure 34) of samples used in genetic analysis.

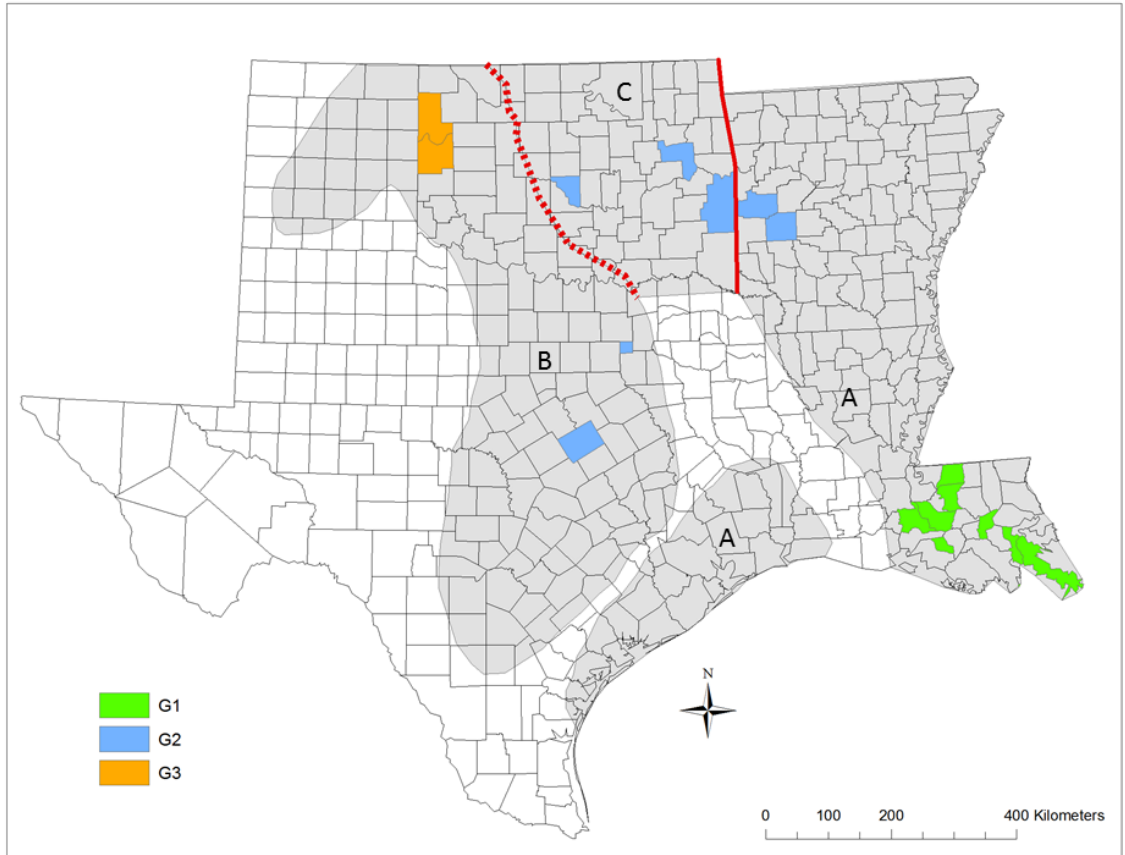


Figure 35. Geographic representation of clades from maximum likelihood phylogeny of the mtDNA gene ND2 for the (A) eastern gartersnake (*Thamnophis s. sirtalis*), (B) the Texas gartersnake (*T. s. annectens*). (C) the red-sided gartersnake (*T. s. parietalis*). Additional data for individuals used in this analysis can be found in Table 4. Gray portions represent the historic range of each subspecies according to Rossman et al. (1996). Red lines indicate range boundaries. Red dashed lines indicate uncertainty of range limits.