USING A HABITAT SUITABILITY MODEL AND MOLECULAR ANALYSES TO AID IN THE CONSERVATION MANAGEMENT OF THE TEXAS TORTOISE, *GOPHERUS BERLANDIERI*

by

Anjana Parandhaman, B.S.

A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Master of Science
in Wildlife Ecology
August 2015

Committee Members:

Michael R. J. Forstner, Chair

Shawn F. McCracken

Thomas R. Simpson

Clay M. Green

FAIR USE AND AUTHOR'S PERMISSION STATEMENT

Fair Use

Duplication Permission

ACKNOWLEDGMENTS

I would like to thank my committee chair and advisor, Dr. Michael R. J. Forstner, for allowing me the opportunity to conduct this research at Texas State University and for his guidance and support throughout the process.

I would also like to thank my committee member Dr. Shawn F. McCracken for his patience and help with answering all of my questions, and my other committee members Dr. Thomas R. Simpson and Dr. Clay Green who provided support and kind words throughout my time at this university. I also extend a word of thanks to the rest of the faculty who guided my education at this research institution.

This thesis would not be possible without the funding provided by Texas Parks and Widlife Department. I would also like to thank my lab mates for their help in the field and the lab, especially Ivana Mali, Andrea Villamizar Gomez, Lauren Schumacher and Andrew Maclaren. I am also grateful for the help that Trina Guerra provided by answering the million lab related questions that I had, and to Prateek Burman for making sure I had enough to eat. Of course the friends I made during my time here provided unending guidance, support and encouragement. Without the help of some of these people I could never have achieved this goal.

Finally, I thank my parents, Vasumathy Parandhaman and Venugopal Parandhaman, for always believing in my abilities and encouraging me to achieve my dreams.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

ABSTRACT

The Texas Tortoise, *Gopherus berlandieri* (Agassiz 1857), is a threatened species in the state of Texas and strict conservation action is required to ensure that continuing population decline does not occur. The historical range of the Texas Tortoise includes a much larger area than recent observations support. Assessing the habitat suitability of the eastern portion of the historical range of the species and determining whether this region still supports the species will aid in its conservation. Firstly, road surveys were conducted from March to October of 2014 and seven tortoises were found during this period. None of these tortoises were from the eastern portion of the range that was the focus of the surveys. GPS coordinates of tortoises from these surveys along with coordinates obtained from online databases were used with environmental predictors to model habitat suitability for the species using ArcGIS (v10.2) and Maxent (v3.3.k). I found that there are some patches of habitat in the eastern portion that could potentially support the species. In addition, I found areas of suitability in far south Texas, as well as in the northern and western regions of their historical range. Secondly, 22 molecular markers in the form of microsatellite loci that were previously found to amplify in *G. polyphemus*, were re-tested for cross-amplification in *G. berlandieri.* Nineteen out of the 22 loci cross-amplified successfully. Seven additional untested markers were further focused on and I found that four of the seven were polymorphic, and had variable levels of allelic richness.

I then performed a population genetics analysis using STRUCTURE v2.3.4 to determine whether tortoises found out of their range fell into a known subpopulation to allow for repatriation and this indicated that *G. berlandieri* has no segregation into multiple populations or clusters. This might be due to low sample size and less markers used or an artifact of most samples being from the same area. These studies attempt to explain the poorly understood factors of habitat suitability, and aid genetic diversity research for the Texas Tortoise. This in turn will allow for better management and conservation of the species throughout its range.

CHAPTER I

INTRODUCTION

The Texas Tortoise, *Gopherus berlandieri* (Agassiz, 1857), is a state threatened reptile in Texas. It is listed in Appendix II under the Convention on International Trade in Endangered Species (CITES) (Groombridge, 1982; Rose and Judd, 1982). However, it is the only *Gopherus* species in the United States that is not federally listed under the United States of America Endangered Species Act. The historical range of the Texas Tortoise includes the Tamaulipan thornscrub and coastal plains ecosystems that extends from Southern Texas to Coahuila, Nuevo Leon, and Tamaulipas, Mexico (Rose and Judd, 1982). In Texas, the species occurs south of a line from Del Rio to San Antonio and Rockport (Fig. 1; Rose and Judd, 1982). This includes Val Verde, Kinney, Uvalde, Medina, Bexar, Karnes, Refugio, and Aransas counties and all counties southward in the state (Rose and Judd, 1982). However, there is also an array of Texas counties where the species has been detected outside this historical distribution. These specimens may have naturally migrated to some of these areas or simply been anthropogenically transported to them (e.g., Brewster, Tarrant, Coleman, Sutton, and Kimble counties; Dixon, 2013).

Less is known about the Texas Tortoise than other North American tortoises, *Gopherus agassizii* and *Gopherus polyphemus*, even though it is the only species of tortoise in Texas. It is the smallest (maximum recorded size = 228 mm) and most sexually dimorphic of the four extant species of genus *Gopherus* (Judd and Rose, 1983; Bury and Smith, 1986). These long-lived herbivores are found in arid or semi-arid

ecosystems, such as the thornscrub ecosystem of south Texas (Judd and Rose, 1989). They are generally found in vegetation loosely characterized as coastal prairie or thornscrub (Rose and Judd, 1975; Rose and Judd, 1982). In coastal areas, Texas Tortoises occur on 'lomas', which are clay to sandy ridges surrounded by salt flats and marshes (Bury and Smith, 1986). The thornscrub ecosystem is mainly dominated by Honey Mesquite (*Prosopis glandulosa*), Granjeno (*Celtis spinosa*), and Prickly Pear (*Opuntia* spp.) (Bury and Smith, 1986).

Texas Tortoises are most active after short periods of rainfall, but if rainfall continues for a period of days, the tortoises become inactive again (Rose and Judd, 1975). Temperature and light also play a role in activity of this species (Rose and Judd, 1975). The preferred body temperature for Texas Tortoises is around 30°C, but never below 22°C (Rose and Judd, 1975). When temperatures reach 40°C, the tortoises become inactive (Rose and Judd, 1975). In April, tortoises are usually active at midday, but by August they have two 'diel' activity periods, morning and afternoon, avoiding high mid-day temperatures in the summer (Auffenberg and Weaver, 1969; Rose and Judd, 1975; Bury and Smith, 1986).

Initially, the Texas Tortoise was considered nomadic with no home range (Auffenberg and Weaver, 1969). Later work revealed that home ranges do exist, with males having larger home ranges than the females (Rose and Judd, 1975). Typically, home ranges for males vary between 0.45 - 2.38 ha and 0.22 - 1.40 ha for females (Rose and Judd, 1975). Home range size is related to food availability, shelter size, sex, age, reproductive condition, and individual variability (Auffenberg and Weaver, 1969; Rose and Judd, 1975). Unlike burrows made by other species of *Gopherus*, Texas Tortoises

use a pre-dug mammal burrow or create shallow pallets (Rose and Judd, 1975). The tortoise uses its forelimbs and shell to scrape an area under the base of trees, shrubs or cacti to create these pallets. The pallets are mostly used during the extreme heat of the summer and the cold during winter (Auffenberg and Weaver, 1969).

Males can be distinguished from females by the presence of a depression in the inguinal region of the plastron, longer gular extensions, longer tails, and larger chin glands (Judd and Rose, 1989). Sex ratios are generally 1 male: 1 female (Rose and Judd, 1982). Females deposit eggs in nests beginning in April and extending till mid-September. Clutch size varies from 1-5 eggs (Judd and Rose, 1989). Eggs are laid in burrows dug by the females. Because the soil can be compact, females sometimes lay eggs on the surface itself (Judd and Rose, 1989). Egg laying is mostly bimodal, with approximately two clutches laid per season. The young emerge a few months after the clutch is laid (Rose and Judd, 1982).

Due to changes in agricultural practices such as livestock grazing and changes in land use including an increase in oil and gas exploration and extraction, there has been a reduction in available habitat for tortoises (Auffenberg and Weaver, 1969). High-volume horizontal hydraulic fracturing (fracking) is a process used to obtain oil and gas, and has become a large industry in Texas. Little is known about the effects of fracking on tortoises. Various effects of fracking on other species, especially those with small geographic ranges, are due to salinization and forest fragmentation caused by this practice of petroleum extraction (Gillen and Kiviat, 2012). Another concern is the development of access roads and significant increases in vehicular traffic that may contribute to increased road mortalities of Texas Tortoises (Auffenberg and Weaver,

1969; Hellgren *et al*., 2000). Large-scale fracking is currently underway in the Eagle Ford Shale in South Texas, and will, or possibly has already affected the Texas Tortoise. In addition, there are over four million acres (1.6 billion hectares) of private ranches in Texas surrounded by deer proof fencing that could potentially impede tortoise movement (Rose and Judd, 2014). Also, the introduction of buffelgrass (*Pennisetum ciliare*) and agricultural practices including grazing and conversion to row crops has resulted in approximately 90% reduction of the brushland in the Lower Rio Grande Valley (LRGV) of Texas since the 1900's (Ramirez, 1986; Jahrsdoerfer and Leslie Jr, 1988).

Despite this, few field studies or sampling efforts have been done across the range of this species. Most studies have focused on protected habitats in southern counties such as Dimmit, La Salle, and Cameron (Judd and Rose, 2000; Kazmaier and Hellgren, 2001). The need for conservation with a better focus on management units and a conservation plan for the tortoise led to this research.

After a review of all available Texas Tortoise observations using a combination of available databases such as iNaturalist, HerpNet, VertNet and the MRJ Forstner Tissue Collection at Texas State University, I found that most sightings were from the southern and western parts of the species' range in Texas (Fig. 1). Only a handful of sightings were from the eastern portion of the range. This is either due to a lack of data or the probable absence of the species from the eastern region. The purpose of my research, was to focus on the eastern portion of the range, determine if the area was still suitable for the species and to delineate areas for conservation. The second part of my research focused on molecular genetics for the Texas Tortoise, specifically testing new microsatellite loci

and running preliminary population genetics analyses. These molecular markers could then be used in the future to implement better conservation plans for the species, specifically within this eastern portion of the range. It would also allow for genetic repatriation of tortoises, found outside the tortoise's historical range, back into its habitat.

CHAPTER II

USING A HABITAT SUITABILITY MODEL FOR CONSERVATION MANAGEMENT OF THE TEXAS TORTOISE, *GOPHERUS BERLANDIERI*, IN THE EASTERN PORTION OF ITS RANGE

INTRODUCTION

To conserve a species one must protect the habitat in which the species resides. To do this one must first know which habitat is suitable for the species (Store and Jokimäki, 2003). Habitat suitability modeling helps predict species distribution and therefore aids in conservation biology efforts. It is a tool for the management of endangered species, ecosystem reintroduction, and population viability analyses (Palma *et al*., 1999; Sanchez-Zapata and Calvo, 1999; Hirzel *et al*., 2001).

Maximum Entropy (Maxent) modeling is a technique for making predictions from presence only data or from 'incomplete' information. Along with presence data, a set of predictor variables such as climatic and topographic variables known to influence the species under study are required (Phillips and Dudik, 2008). Maxent estimates a target probability distribution by calculating the probability distribution of maximum entropy (i.e. most spread out) (Phillips *et al*., 2006). It is a method by which estimations of uniform distribution of sampling points are made as compared to background locations, given constraints in the data (Grendar and Grendar, 2001; Phillips *et al*., 2004; Phillips *et al*., 2006). The ability to use presence only data is useful when there is no absence data available, especially when available data is from museum or herbarium collection observations (Phillips *et al*., 2004).

Maxent was chosen as it outperforms similar methods using presence only data (Anderson *et al.,* 2006). It has been used widely to model species distribution, predict species richness, to understand environmental correlates for species, and test model performance (Anderson *et al.,* 2011). It has been widely used by government agencies and other organizations for various projects such as the Point Reyes Bird Observatory online application (http://www.prbo.org/).

The purpose of this portion of my research was to assess the current habitat suitability for the Texas Tortoise within its historic range, particularly in the eastern portion of its range, using Maxent (version 3.3.3k; http://www.cs.princeton.edu/~schapire/maxent/; (Phillips *et al*., 2004 and 2006) and ArcGIS (v 10.2; ESRI, 2013) modeling tools.

OBJECTIVES

The objectives of Chapter II include:

- Creating a database of all existing literature pertaining to the Texas Tortoise in Texas and making this publicly available on Mendeley (a free program for managing and sharing research papers).
- Obtaining all existing location information on Texas Tortoise, but not shared on Mendeley (GPS coordinates).
- Conducting systematic road surveys in the poorly known eastern portion of the range of Texas Tortoise, representing approximately 25 percent of the known range of the species and obtaining observation data in the form of GPS coordinates for any tortoises found.

- Creating a habitat suitability model using Maxent and ArcGIS software based on tortoise locations from road surveys as well as data from online databases. Land cover data and other relevant environmental parameters will be used to assess most suitable areas for the species.

METHODS

Mendeley Reference Manager

I conducted an initial literature search pertaining directly to studies conducted on the Texas Tortoise (*G. berlandieri*) using library resources at Texas State University, Google Scholar, and Web of Knowledge search engines. I added citations of literature pertaining to the Texas Tortoise to an online bibliography hosted at the free Mendeley Reference Management site (www.mendeley.com) under the group name: *Gopherus berlandieri*.

Texas Tortoise Surveys

I conducted road surveys monthly from March through October of 2014, to search for live and dead tortoises along roads. Primarily, I focused these surveys on areas to the east of State Highway 16 and north of State Highway 285. I conducted additional road surveys along the northern species boundary, roughly along a line from San Antonio to Del Rio. These surveys were conducted for approximately 12 hours a day, generally starting shortly after sunrise with a midday break and then continuing until sunset. Potential rain events in the study area were specifically prioritized for survey trips. Additionally, surveys were conducted by means of line transects on the James E. Daughtrey Wildlife Management Area, in McMullen County, on October 12, 2014. Potential burrows/pallets were observed but no tortoises were detected.

In addition to data required by the Texas Natural Diversity Database reporting form, I collected weight, carapace length and width, plastron length and width, body depth, and sex for each tortoise encountered. Each tortoise was photographed in dorsal, ventral, anterior, posterior, and lateral left and right aspects. I assigned each live tortoise a unique identification code at time of first encounter using a dremel tool to incise marginal scutes (Cagle, 1939). These ID codes allow for recapture identification. From live tortoises, I drew a small aliquot of blood (~1mL) from the femoral vein and placed in blood storage buffer (IACUC Protocol # 0417_0513_08). From dead tortoises, I collected muscle tissue from the least-exposed area of the carcass and placed in 95% ethanol. Blood and tissue collections were stored at -80ºC in the MRJ Forstner Tissue Collection at Texas State University.

Texas Tortoise Observation Data

In addition to our survey data observation points, I conducted extensive database searches for Texas Tortoise observational geodata using the following databases: MRJ Forstner Tissue Collection at Texas State University, Biodiversity Information Serving Our Nation (BISON), VertNet, iNaturalist, Texas Natural Diversity Database (TXNDD), and unpublished field data of Dr. Francis Rose (Professor Emeritus; Texas State University).

I created three datasets of observation points based on accuracy of the location data. The first had all possible coordinates (610 points) with an accuracy of less than 10 miles. The second dataset had coordinates with less than 500 meter accuracy (251

points). The third dataset had coordinates with less than 10 meter accuracy (180 points) (Table 1).

Pre-processing of Data

Environmental variables, known to affect the presence of the Texas Tortoise, were determined from the literature and expert consultation (Andersen and Beauvais, 2013; Rose and Judd, 2014). I used the Texas Tortoise model built by Andersen and Beauvais (2013) as a primary reference for this purpose. For their model, TXNDD biologists were consulted to determine environmental variables that affected Texas Tortoises. I used similar variables, and incorporated additional variables we deemed appropriate to the study. All processing was done using ArcMap (10.2).

1. Climatic variables

I obtained climate data from www.worldclim.org in the form of 30 arc-second cell size ESRI-rasters (Hijmans *et al*., 2005). Six predictor variables- Mean Diurnal Range, Isothermality, Minimum Temperature of Coldest Month, Mean Temperature of Warmest Quarter, Precipitation Seasonality, and Precipitation of Warmest Quarter were selected based on the model built by Andersen and Beauvais (2013) (Table 2).

I pre-processed these variables using ArcMap and converted to ASCII files for use in Maxent. These variables were checked for multicollinearity using the SDM Toolbox available from http://sdmtoolbox.org/ (Brown, 2014). All six climatic variables

expressed high collinearity (>0.7). Four variables showed a collinearity greater than 0.8, which was then used as the cut off to determine the variables to be used. Two variables (Bio15 and Bio6) showing the least collinearity (<0.8 but >0.7) were used for further analyses (Figs. 4a-4b).

2. Land Use and Land Cover

For land use and cover, I used LANDFIRE existing vegetation cover dataset (http://www.landfire.gov/datatool.php) and the National Land Cover Database (NLCD) (http://www.mrlc.gov/nlcd2011.php). LANDFIRE (2012) data was used to create five variables: shrub canopy cover, forest canopy cover, herb cover, agricultural land, and development (Figs. 2a-2d; Figs. 3a-3b). NLCD (2011) data was used for one categorical variable: Percentage Forest Canopy Cover (Fig. 2d), in addition to the LANDFIRE forest canopy cover layer. I considered additional vegetation type data from LANDFIRE for analysis, but accurate classifications could not be attained for use in modeling.

I created the NLCD layer by reclassifying the Percentage Forest Canopy Cover layer into a categorical layer with Deciduous Forest, Evergreen Forest and Mixed Forest being classified as 1 and remaining types classified as 0.

I created the Agricultural Lands layer by reclassifying the Existing Vegetation Cover data from LANDFIRE 2012 into a categorical layer. NASS Row Crop-Close Crop, NASS Row Crop, NASS Aquaculture, and NASS Vineyard were reclassified as 1 and remaining types as 0.

I created the Shrub Cover layer, Herb Cover layer and Forest Cover layer, from LANDFIRE 2012, by reclassifying based on the midpoints of the percent cover estimates for each level of vegetation (Andersen and Beauvais, 2013) (Table 3-5).

In addition, I was interested in looking at the effects of development on the tortoise, so I created a categorical development layer and reclassified Developed-Low Intensity, Developed-Medium Intensity, Developed-High Intensity, Developed- Quarries, Strip Mines, and Gravel Pits as 1. Remaining types were classified as 0.

3. Soil

Soil layers were obtained from the Gridded SSURGO (gSSURGO) database (http://datagateway.nrcs.usda.gov/**).** I selected Percentage Sand and Saturated Hydraulic Conductivity (ability of moisture to move through the soils in micrometers per second) as the two predictor variables based on their use by Andersen and Beauvais (2013). However, since the Texas Tortoise uses pallets and other burrows that are relatively shallow (pallets less than their carapace length), I decided to not use the entire soil layer depth but rather just the surface soil (Auffenberg and Weaver, 1969). I obtained the dominant soil component for the surface soil of each soil map unit with help from NRCS staff (Amanda Bragg, pers. comm.). These data layers represent the upper soil surface layer (<30 cm) of the soil and rainfall layer (Figs. 3c and 3d).

All of the above layers were projected in the USA Contiguous Albers Equal Area Conic USGS projection, converted to 30 meter pixel resolution, and then converted into ASCII format for input into Maxent.

Maxent Model Building

I used Maxent for modeling the species' distribution. Three different sets of observational data (Table 1) were used to build various models. I used all 14 environmental variables to run a model with a subset of coordinates. However, I found that most variables were correlated with a Pearson's Correlation Coefficient > 0.7. Subsequently, I conducted analyses using just 10 of the 14 variables, removing highly correlated variables. Furthermore, I ran models in iterations with the development layer and without, as inspection of the layer showed a sampling bias towards roads. Latitudinal bias was corrected for by using a bias file created using SDM tools (v1.1c) (Brown, 2014).

Up to three replicates were run for each model, using subsampling, cross-validation, and bootstrapping for model validation. These replicates were averaged with a mean, standard deviation, minimum and maximum values of probability across all runs. Subsampling was chosen as the best model validation method as it is generally used for medium to large datasets, as compared to cross-validation and bootstrapping.

I also performed model validation by randomly selecting 20% of the sampling points (sample coordinates used) as a test dataset, to run separately from the remaining 80% of the dataset to be used as training data to build the final model. The Area Under the Curve (AUC) was calculated for the test dataset to predict model accuracy (Fielding and Bell, 1997). Also, Maxent creates background or 'pseudoabsence' points to model distribution of a species. These points help distinguish between areas 'used by' versus

those ‘available to’ the species (Andersen and Beauvais, 2013). Around 10,000 random background points were created for this purpose representing all gradients available to the species.

I performed a jackknife analysis on all environmental variables in the models, where one predictor was withheld and the model was refitted. This was done to determine the contribution of each variable to the model. The other model settings used were as follows: Feature types used: hinge linear quadratic; responsecurves: true; jackknife: true; randomseed: true; writeclampgrid: false; writemess: false; randomtestpoints: 20; writebackgroundpredictions: true; replicates: 3; replicatetype: subsample. The agriculture, development and NLCD-Forest cover layers were treated as categorical layers in Maxent. In total I ran 18 models using the various model validation layers (cross-validation, bootstrapping, and subsampling), the three different sets of observation points, different iterations, and exclusion of various variables. The final chosen subsampling validation method was used to run models with variables iteratively removed. These were then used for further analysis and interpretation (six models in total).

I selected the best fit model by calculating AIC_C using ENMtools (v 1.4.4) run using a Perl script (Warren *et al.*, 2010). I re-ran models to create output in ‘raw’ format for input into ENMtools. I calculated AIC_C to compare each replicate of every model built, as well as to compare the various models themselves.

All output was logistic and in the form of ASCII files. I then imported these files into ArcMap to create final maps.

RESULTS

Mendeley Reference Manager

I created a User Group (=*Gopherus berlandieri*) on the free online Mendeley Reference Manager (http://www.mendeley.com/groups/4224511/gopherus-berlandieri/) to produce a bibliography of all literature pertaining to the Texas Tortoise. The *Gopherus berlandieri* Group currently contains 70 literature citations that directly pertain to the species and is continuously being updated as new literature is discovered.

Texas Tortoise Surveys

Approximately 17,816 miles (28,672 kilometers) of roads were surveyed from March to October of 2014, representing the eastern, southeastern (coastal), northern and northwestern portions of the Texas Tortoise range (Fig. 5). These survey efforts represent a total of approximately 900-1000 person hours. In total, only 7 tortoises were found during these surveys (Fig. 6). Four of these tortoises were road mortalities; the other three were alive and were marked and released. Other tortoises were found during the same time period outside of the range being surveyed and were included in our final model. Most were suspected to be released pet tortoises or human translocated individuals outside their range, but a few were from the northeastern boundary for the species and may represent wild individuals (Fig. 7).

Texas Tortoise Observation Data and Maxent Model

I identified over 600 coordinates from database searches and unpublished data such as VertNet, BISON, iNaturalist, and the MRJ Forstner Tissue Collection at Texas State University. I then divided them into three categories based on the level of accuracy while collecting the points (Table 1). Modeling was carried out using Maxent.

One way to measure fit or accuracy of the model produced through Maxent is by Receiver Operating Characteristic Plots (ROC) (Baldwin, 2009). An ROC plot measures sensitivity and specificity of the data. Sensitivity measures how well the data predicts presence, whereas specificity measures correctly predicted absences (Fielding and Bell, 1997). I developed the ROC plot by using separate training and testing data for each replicate and averaging them. The plot can be read by looking at the Area Under the Curve (AUC); in this case the high AUC of all six models indicates a perfect fit that is better than that expected by random chance (Baldwin, 2009). Model one, with 180 points and 10 variables and model four with 180 points and 9 variables had the highest AUC's of 0.966 and 0.976, respectively (Table 6). Models two, three, five and six, had lower AUC's (0.949, 0.926, 0.954, 0.929, respectively). These models had sample points with higher uncertainty in data collection, despite having a greater number of data points and being more representative of the species historic range. Many studies tend to choose the model with the highest AUC as the best fit model but in recent studies this has been found to be inaccurate, as AUC tends to choose the models with the most parameters (Warren and Seifert, 2011). Therefore, a better estimate of model selection- AIC_c was

calculated for the above six models using ENMTools. In addition, the AIC and BIC values were also calculated.

Models one and four that had the highest AUC's (0.966 and 0.976) also had the lowest AIC_C's (5146.5 and 5298). The other models had very high AIC_C's. However, model one had the lowest ΔAICc and I chose this as the best fit model and subsequent results were based on this model (Table 7).

The first output produced in Maxent is the analysis of omission/commission that evaluates model performance/bias, as a function of predicted occurrence. It displays the omission rate and predicted area at different thresholds (Young *et al*., 2011) (Appendix II). The omission rate should be close to the predicted omission, and when compared to other models the best fit model had the closest omission rate.

Environmental variable importance can be assessed in two different ways. First, Maxent provides the percent contribution and permutation of importance for each variable used in the model. These are calculated by determining the increase in gain by each variable in the model. This can be seen for the selected model in Appendix II. Variables bio6 (Minimum temperature of coldest month) and bio15.2 (precipitation seasonality) had maximum contribution and permutation importance followed by the developed land categorical variable. The second is a jackknife analysis performed on the variables (Appendix II). This excludes one variable at a time while running the model to estimate performance of each variable, based on gain (Baldwin, 2009). Jackknife analyses on gain of training data, gain of test data, and also on AUC were created. These jackknife analyses show that the gain for bio6 is the maximum when looked at in isolation. Similar results can be seen in all jackknife analyses

performed. This indicates that bio6/minimum temperature of coldest month, followed by precipitation seasonality/bio15 and then percent sand have highest predictive power for determining distribution of Texas Tortoise (Appendix II). However, when looking at the response curves one can see that more developed areas have higher probability of occurrence of tortoises. This indicates a slight skew in the data as many of my presence locations were collected near roads and inhabited areas due to inherent observer bias towards developed areas. Also, forest canopy cover had the least contribution to the model.

As stated before, Maxent also produces response curve outputs (Appendix II). I created the first set of response curves by treating each variable in isolation and then averaging the rest of the variables. I created the second set of response curves by developing a Maxent model for each response variable separately. These curves indicate probabilities of occurrence of the tortoise in relation to each variable used in the model.

Looking at the model itself one can see areas showing high probability of presence in far south Texas, along the Mexico border (Fig. 12). Areas indicated by red can also be seen further north and towards the west. When examined, the area with high suitability towards the west of the range was in and around the Chaparral Wildlife Management Area, in La Salle and Dimmit Counties. Furthermore, the model indicates areas of suitability in the eastern portion of the range that aren't as high as other portions of the range. A scattering of probability of presence can also be seen outside the range of the species (Fig. 13). I included models two and three as comparison to the model that was selected (Figs. 16 and 17).

DISCUSSION

Mendeley Reference Manager

Mendeley has the potential to benefit future research and create a community of researchers. Currently, there are more than 70 Texas Tortoise literature citations on Mendeley, with additional citations being added as they become available. This can now be used as a common platform to access studies on the species by subsequent researchers. Users and myself will continue to update the list as more literature on the species is obtained. One of the features of Mendeley is that Group users (=*Gopherus berlandieri*) can access and update the citations, allowing for perpetual use and updates to the existing literature which also enables networking among researchers involved with the Texas Tortoise.

Texas Tortoise Surveys

In the time frame of my study I found a total of 7 tortoises, but none of these were in the eastern or northern portions of the range. In comparison, the number of iNaturalist observations we obtained for the same time period was just 9 and again none of those were from the eastern or northern portions of the range, despite there being many more people potentially contributing to the database compared to my research group. This indicates either the species is not as prevalent in the eastern portion of the range as other areas, or that more intensive surveying needs to be carried out. The

results of my road surveys and the data obtained from iNaturalist are particularly disconcerting when compared to historic road survey results from Hamilton (1944), when he observed 16 individuals along 2-3 miles of highway in between Bee County and San Patricio County, and a similar number just off the highway after a rain on August 3, 1938 further south in Texas.

Habitat Suitability Modeling

My chosen model (Figs. 18-19) revealed areas of high suitability (probability of occurrence >0.6) in many parts of South Texas as well as areas further to the north. There are some areas of my model in South Texas, where linear features of very high suitability as well as in areas of higher human habitation that I interpret as artifacts of tortoises being observed in greater numbers where more people occur. It is interesting to note that there are a few suitable areas in the eastern region where tortoises are not encountered as frequently, and similarly to the northwest. There are areas outside the suitable habitat where tortoises were found, and this adds support to the fact that these tortoises were most likely either relocated or longer term "pets" released into the wild.

In conclusion, I can state that despite not finding any tortoises in the far eastern or northern range of the species, my model indicates that suitable habitat patches do remain present in these regions. This is also true further outside the range, where areas of potential suitability just north of the generally accepted range in Gonzales, Dewitt, Victoria, Hays, and even Travis County (Fig. 13) can be seen. However, my environmental layers are not representative of ongoing landscape changes due to the

high level of renewed oil and gas operations and their supportive infrastructure in the Eagle Ford Shale of South Texas since 2008, particularly in the eastern and western portion of the range (Fig. 14). I am also unable to account for the effects of significant vehicular traffic increases on the remaining Texas Tortoises within this same area. These areas will require extensive targeted survey efforts to more clearly validate the accuracy of the model, but extensive surveys are hampered by the large private land holdings that limit access to much of the potential tortoise habitat identified. Another limitation of my surveys was the relatively short amount of time I had for survey validation efforts, being limited to less than a single season of potential tortoise movement that hampered detection. In addition, other potential areas of suitability delineated by my model can be explicitly targeted for future studies guided by the current model. This can help add to 'absence' data that in turn will help with reiterative model building and performance, potentially using other modeling programs as well.

My model differs from the model produced in the study by Andersen and Beauvais (2013). First, I used fewer environmental variables (10 versus 13) to reduce multicollinearity, and the values for my soil layers were restricted to the upper 30 cm of soil depth compared to the full horizon depth used by Andersen and Beauvais (2013). I also used other variables I thought might influence presence of the species. Second, I used more presence locations (up to 612 sampling points versus 60 sampling points) and applied 20% of my points as test data to validate my model. Third, the sampling points I used were of higher accuracy up to 10 meters versus <8000 m. Fourth, my model also indicates novel patches of suitability, when contrasted to their final model, especially in the northwestern and eastern areas of the range (Figs. 12 and 13). Finally,

my selected model does not generate the areas of mid-level predicted suitability in areas of north and west Central Texas predicted by theirs (Anderson and Beauvais, 2013).

The percent contribution of the environmental variables lends support to what we know about Texas Tortoises. The Tamaulipan Biotic Province is characterized by hardly any forest canopy cover and thick brushland (Rose and Judd, 2014). My model had the least contribution by the two forest canopy cover variables, indicating that tortoises are not influenced by presence of forest canopy cover.

Future model development will include the use of more presence locations as more coordinates are obtained for the species from my data sources and future surveys. Ground truthing of suitable areas must also be carried out. Additionally, on inspection it was found that tortoises are found in high numbers in areas with Duval type of soil. This variable could not be accurately incorporated into the modeling process, but future steps will be undertaken to look at the potential impact this individual variable might have on the overall suitability for species occurrence.

The greatest success of my model is in its ability to enable detection for areas of greatest concern and the factors impacting the species so that conservation management efforts can be engaged in the remaining suitable habitat patches. However, caution must be exercised when using such graphical depictions of habitat suitability due to contemporary impacts on the landscape that are not reflected in these models. I have illustrated several concurrent anthropogenic landscape alteration activities within the range of the Texas Tortoise that are rapidly changing the habitat of South Texas. The wildfire, in 2008, at the Chaparral Wildlife Management Area destroyed over 95% of habitats used by the species on the property. This potentially affected over 90% of

tortoise populations (Berry and Aresco, 2014). However, no studies have been published that examine the population status of the Texas Tortoise after the event. Bury and Smith, in 1986, suggested that prescribed fires could improve open areas available to tortoises. In Desert Tortoises (*Gopherus agassizii)* it was found that wildfires destroyed vegetation and led to potential dietary changes in the species (Esque *et al.,* 2003). Until we are able to gain a better understanding of the effects of ongoing and increasing disturbances in the region, and considering my very low number of observations under extensive survey effort, it is imperative that continued progress be made toward the protection of the Texas Tortoise.

CHAPTER III

WHERE AM I FROM? A GENETIC ASSESSMENT OF TEXAS TORTOISES ACROSS AND OUTSIDE THEIR RANGE

INTRODUCTION

Conservation of a species is aided by looking at the underlying genetic diversity of the species. Low genetic diversity or low average heterozygosity indicates that populations of the species might not be viable. These populations might experience increasing genetic drift with decreasing genetic diversity. They might not be able to cope with a changing environment, as their average heterozygosity is low (Franklin, 1980; Frankel and Soule, 1981; Frankham *et al*., 2002; Fujii and Forstner, 2010). Disturbances to habitat, which is a huge threat to the Texas Tortoise (Rose and Judd, 1982; Judd and Rose, 1989; Judd and Rose, 2000) could result in fragmentation of gene flow. Habitat fragmentation may lead to increased allelic loss and decrease in average heterozygosity, which might lead to extinction of the species (Cunningham *et al.,* 2002). As stated before, fragmentation reduces viability of a species, and so assessing all of these factors will aid in the creation of a management plan for protection of the species.

Conservation genetics of tortoises broadly includes studies on systematics and phylogenetics (Lamb and Lydeard, 1994), population structure (Fujii and Forstner, 2010), phylogeography (Osentoski and Lamb, 1995) and hybridization (Edwards *et al.,* 2010). There has been an increase in recent times in the utilization of microsatellite or short tandem repeats (STRs) to undertake conservation genetics studies of tortoises (Edwards and Harrison, 2014). These STRs are inherited from both parents and have a faster rate of evolution than mitochondrial DNA. They also allow for sampling across the genome,

thus allowing for better estimates of demography and gene flow (Edwards and Harrison, 2014).

In the past, there have been very few studies done on molecular genetics of *G. berlandieri.* Fujii and Forstner (2010) looked at the population structure of *G. berlandieri* and determined that there are two populations, one to the north of the Nueces river and one to the south, defined by a boundary at southern Duval County. Two other studies have tested various microsatellite loci in other *Gopherus* species, for cross-amplification in *G. berlandieri* (Kreiser *et al.,* 2013; Schwartz *et al.*, 2003). This lack of data warrants the need for studies in the field of molecular genetics for the Texas Tortoise to aid in management and recovery plans.

The second part of this study involves testing previously known microsatellites for the Texas Tortoise. Kreiser *et al.* (2013) identified 32 polymorphic microsatellite loci in *Gopherus polyphemus*. Of these 32, 29 were tested for cross amplification in *G. berlandieri*, and 22 microsatellite loci were amplified (Kreiser *et al.* 2013). The current study will identify new microsatellite loci that were previously untested in Texas Tortoise by Kreiser *et al.* in 2013. These loci were found to successfully amplify in *G. polyphemus* (Kreiser *et al.*, 2013).

In the previous studies by Fujii and Forstner in 2010, only two samples collected from the eastern portion of the range of the species were used. This study will also strengthen the previously existing data by using all tortoise samples obtained from the eastern region during the 2014-2015 sampling period.

Also, during my field studies we obtained a large number of released pet tortoises or human translocated tortoises. Determining the population substructure for these tortoises would aid in repatriating these individuals back into their habitat.

METHODS

Microsatellite Genotyping

I extracted genomic DNA of tortoise blood and tissue samples, obtained during 2014-2015, following the DNeasy kit protocol (QIAGEN Inc., Valencia, CA).

Twenty-nine microsatellite loci developed by Kreiser *et al.* (2013) were synthesized with a 5' M13 tag attached to the forward primer (Integrated DNA Technologies, Coralville, Iowa) (Schuelke, 2000). Seven of these 29 loci were previously untested in *G. berlandieri*, but all were tested and amplified in the Gopher Tortoise, *Gopherus polyphemus*. I carried out Polymerase Chain Reaction (PCR) on the above 23 samples plus already extracted template DNA from 41 other samples, across these 29 loci. I performed PCR reactions on a Peltier Thermal Cycler in 25µl reactions consisting of 5X *Taq* reaction buffer (GenScript), 0.5-1.0 mM added $MgCl_2$, 50µM dNTPs, 0.04 units of *Taq* polymerase, 0.16µM of the reverse primer, 0.16µM of the M13 labeled primer, 0.1µM of the M13 tailed forward primer (Schuelke, 2000), Bovine Serum Albumin (15mg/ml), 20-100 ng of template DNA, and water to the final volume. PCR cycling conditions were as follows: an initial denaturing step of 94°C for 2 minutes, followed by 35 cycles of 30 seconds at 94°C, 1 minute at 48-56°C, and 1 minute at 72°C, followed by a final elongation step at 72°C for 10 minutes that ended the cycle (Kreiser *et al*., 2013). I varied PCR conditions and reagents as necessary per locus and also when the universal fluorescent labeled M13 primer was added (Schuelke, 2000).

I undertook an initial PCR analysis of all 29 loci and 24 of these loci showed amplicons. Further analysis was conducted with only the 7 untested markers. I used only four loci out of these 7 markers for further analysis based on amplification with the fluorescent label. I analyzed these using an ABI 3500xL Sequencer with a 35-500 bp size standard. I visually inspected and binned peaks using GeneMapper (v4.1).

Statistical Analyses

I used CREATE (v1.37) to format the genotyping results correctly so that I could input them into various programs for analysis. I used MICRO-CHECKER (v2.2.3) to detect the presence of null alleles, genotyping errors due to scoring of stutter peaks and large allele dropouts (Van Oosterhout *et al*., 2004). I tested for deviations of the loci from Hardy Weinberg Equilibrium (HWE) using ARLEQUIN (v3.5.2) (Excoffier *et al*., 2005) and 1,000,000 MCMC repetitions. I used a sequential Bonferroni correction to correct α values that did not follow HWE (Rice, 1989). I also used ARLEQUIN to test for pairwise linkage disequilibrium using 10,000 permutations.

I used the program STRUCTURE (v2.3.4) to determine presence of subpopulations, by running 100,000 Markov Chain Monte Carlo (MCMC) estimations and 10,000 burn-ins (Pritchard *et al.*, 2000). I estimated the number of subgroups (K) by running twenty replicates of K = 1-5. I also calculated mean log likelihood as well as the mean genotype proportions of individuals for each K. As the log likelihood is found to be an inadequate method in determining population structure, I used STRUCTURE

harvester to calculate ΔK (rate of change in log probability of successive K values) (Evanno *et al*., 2005; Earl and vonHoldt, 2012).

RESULTS

Microsatellite Genotyping

In total 29 microsatellite loci were tested. Out of 22 microsatellite loci that were known to amplify in *G. berlandieri* (Kreiser *et al*., 2013), 19 showed amplification during PCR, at varying levels (Table 14). I used only seven microsatellite loci for further analyses as these represent loci that were untested in Texas Tortoise previously. Of the remaining seven untested microsatellite loci, five showed amplification, however, one of these five had to be excluded from further analyses as reasonable amplification could not be attained with the M13 fluorescently labeled tag.

In total, 64 samples and four microsatellite loci were visualized on the ABI 3500xL Genetic Analyzer. However, I subsequently excluded samples in which less than 75% of loci were not amplified. This left a total of 55 samples for input into statistical programs.

These samples were from parts of the southern portion of the range of the species, areas towards the northern boundary of the range and also areas outside the range (Fig. 22). The samples obtained from outside the range were possible pet releases or translocated tortoises. In addition, one sample was also obtained from Tamaulipas, Mexico. The eastern and northern regions were represented by one sample from Live Oak County, and a few from Bexar and Comal County (Fig. 22).

Statistical Analyses

All four microsatellite loci that were used for analysis had less than 5% missing data (Table 8). MICRO-CHECKER v2.2.3 (Van Oosterhout *et al*., 2004) did not find any evidence of homozygote excess or scoring error due to stuttering or null alleles. I did not detect large allele dropouts for any of the four loci.

All loci showed polymorphism with 2-10 alleles per locus, and a mean of 4.25 (Table 8). The size ranges for the alleles varied from 3-33 with a mean of 12.75 (Table 8). Two out of four microsatellite loci were found to have higher expected heterozygosity as compared to observed heterozygosity (Table 8). Locus GOPOA111 violated Hardy Weinberg Equilibrium but I used a sequential Bonferroni adjustment to correct α values. Linkage disequilibrium was also noted between loci GOPOA012 and GOPOA111, and again I used a sequential Bonferroni adjustment to correct α values.

Analysis using STRUCTURE showed a mode at K=2 (Ln = -387.34) that was different from K=1, 3, 4 or 5 (Fig. 18). I used ΔK to verify K = 2 using STRUCTURE Harvester (Evanno *et al*., 2005; Earl and vonHoldt, 2012), and found a mode at K = 2 (Fig. 19). However, when I examined the mean genotype proportions, obtained through STRUCTURE, at K=2 I found that there was an equal probability of each individual being assigned to both clusters (Fig. 21). The mean genotype proportions for K=1 was subsequently examined, and I found that all individuals had a probability = 1 of being assigned to K=1 (Fig. 20).

DISCUSSION

The main goal of this chapter was to test microsatellite loci that were previously untested in *G. berlandieri* and obtain descriptive data on genetic diversity for the species. My study also attempted to run some basic population analyses to aid in the repatriation of wayward tortoises.

There were multiple issues encountered with obtaining the required amplification during PCR's of various samples. This might have been due to PCR inhibitors, contaminants, mutations in annealing sites (null alleles), and non-specific binding. Re-extractions and dilutions of template DNA had to be carried out for many samples. When compared to the protocol followed by Kreiser *et al.,* 2013, this study varied significantly in terms of conditions and reagents used through the process. Kreiser *et al.* (2013), successfully amplified 22 primers in *G. berlandieri*, but this study managed to amplify only 20 out of the 22. There were issues with the M13 fluorescently labeled tag as there probably was competition between it and the 5' M13 tagged forward primer. I ran a nested model to determine if the reactions could be carried out separately, but that was unsuccessful as well. Further analyses only included the loci that worked successfully with the M13 fluorescent label.

When compared to Fujii and Forstner's (2010) results, the allelic richness and mean heterozygosity of the various loci in my study fell within the same ranges. Mean heterozygosity was between 0.47-0.53 depending on the location of the population, and mean allelic richness was between 2.0-9.5 (Fujii and Forstner, 2010). My results found the mean observed heterozygosity to be 0.4643 and mean allelic richness to be 4.25.

This corroborates the appreciable amount of genetic variation that Fujii and Forstner (2010) found in *G. berlandieri.*

Analysis using STRUCTURE suggested that a K = 1 is the best fit for the 55 samples that I analyzed, based on examining the mean genotype proportions of individuals at K = 1. In other words, there seems to be no suggestion of multiple populations of *G. berlandieri* as indicated by Fujii and Forstner (2010), who suggested the existence of two weakly differentiated populations for the species. Pritchard and Wen (2010) said that when there is no population structure, the mean genotype proportions will be symmetric, and there can be no biological interpretation of such results. The true K could not be identified by observing Delta K and this is called the absence of signal (Evanno, 2005). This was determined to be due to the reduced number of loci (four) used and low number of samples that were genotyped (55) as compared to Fujii and Forstner (2010) who used 138 samples and eight loci. Delta K can also not be calculated for a K of 1. Looking at the samples themselves we can see that most were from around or north of Duval County, the boundary which according to Fujii and Forstner (2010) is where a weak population structure exists for Texas Tortoises. Knowing this, it was expected that all samples would fall into one of the two subpopulations. However, an exact representation of population structure can only be determined with more extensive sampling across the range and use of additional markers.

The microsatellite novel loci identified to be polymorphic in Texas Tortoise in this study, have potential future uses in various studies. The full extent of variability in genetics of the Texas Tortoise has not been assessed yet. According to an unpublished

study based on Fujii and Forstner (2010), the mtDNA of *G. berlandieri* shows low levels of within population variability (Edwards and Harrison, 2014). One of the samples used in this study was from Mexico, thus possibly expanding our knowledge of the species outside Texas (Fig. 22). My results show that only one out of four microsatellite loci had relatively high allelic richness; 10 alleles as compared to two or three (Table 13).

Future studies should address anthropogenic impacts on the species. As mentioned in Chapter II, oil and gas exploration and extraction is increasingly becoming a threat within the Eagle Ford Shale cutting across prime tortoise habitat. Over 32,000 roads cut across south Texas and this might have a deleterious effect and impede movements of tortoises across the landscape. In addition, there exists over four million acres of deer proof fencing in these areas (Rose and Judd, 2014). This might restrict gene flow and potentially lead to reduced genetic diversity for the species. Population genetic studies could help evaluate impact of such activities on tortoise populations.

These loci could also be used to add to the existing data analyzed by Fujii and Forstner (2010), and examine if the estimated population differentiation for the species between the northern and the southern parts of Texas is 8.3% or if it varies with additional markers. I can also attempt to examine whether the population is in decline especially within the eastern region of its range. Future genetic studies can help contribute to potential recovery units for the species if necessary. Knowledge of genetic diversity and variation, as well as population structure will help in the conservation of the Texas Tortoise across its range.

CHAPTER IV

CONCLUSIONS

In Chapter II, I focused on developing a suitability or species distribution map for the Texas Tortoise, and examined whether there are areas potentially suitable for the species outside its range. I looked at the understudied eastern portion of its range, that historically is part of its range, but contemporarily has not had many sightings for the species. Road surveys were conducted in this eastern portion of the range, but no tortoises were found in this area during the study period. Coordinates obtained from other areas further south, along with coordinates from museum and online databases, were used along with environmental variables to model predicted distribution of the tortoise across Texas. I found patches of suitable habitat across its range, with highest probability in deep south Texas and a few areas further north. Furthermore, areas within the eastern portion of the range had a moderate probability of presence, indicating that the species could potentially be present there despite lack of data from road surveys. Also, areas outside the historic range might harbor the species, possibly a reason for various sightings of tortoises from these areas. The value of this model is in identifying and delineating areas for conservation of *G. berlandieri.*

In Chapter III, I focused on testing microsatellite loci that were previously untested in *G. berlandieri.* Various loci were identified for testing from studies of *G. polyphemus* (Kreiser *et al.,* 2013). Four out of seven loci were cross-amplified

successfully in Texas Tortoise. Other microsatellite loci (22 in total) were also tested and showed varying levels of success. Analysis of population structure showed the presence of one population for the species.

Microsatellite loci can be useful for determining population structures and subdivisions, understanding genetic diversity and determining reductions in population sizes (Edwards and Harrison, 2014). With more microsatellite loci and samples used this can be applied towards conservation of the Texas Tortoise, especially in the poorly studied eastern portion of its range.

An increase in habitat fragmentation, caused by building roads, increasing high fences and other types of development will only contribute to the restriction of gene flow for the species. Tortoises have a long lifespan and produce one or two clutches a year (Rose and Judd, 2014), so any changes to their populations could have long lasting consequences. At present, *G. berlandieri* is probably protected to a certain extent in isolated pockets of private landholdings (Rose and Judd, 2014). High fences impede movements of these tortoises across these lands. This poses a risk to the overall population status, in terms of genetic diversity, and at present this risk is unknown.

In 1994*,* the USFWS proposed six recovery units for the federally listed Desert Tortoise, *G. agassizii*, across its habitat, based on data related to habitat, genetics, morphology, and behavior (Murphy *et al.,* 2007). These units were, as per Murphy *et al.* (2007), redefined to reflect better genetic differentiation at the regional scale. Similarly, Schwartz and Karl (2005) reported eight breeding units for *G. polyphemus*, but Clostio *et al.* (2012) reported the existence of five genetic units. For *G. berlandieri,* studies such as those in Chapter I and III attempt to understand what is poorly understood regarding

habitat, and genetic diversity. This in turn will allow for better management and conservation of the species. If necessary, the creation of such recovery units as in *G. agassizii* and *G. polyphemus* can be implemented once the level of need is established.

The Texas Tortoise is currently listed as threatened at the state level. Little is known about population status across its range and without these data it is impossible to adequately assess if there is a need for more stringent laws. Despite being threatened, there has not been adequate enforcement of rules (Rose and Judd, 2014). At present, studies need to be undertaken not only across the species range in Texas but also into Mexico, where even less data is available (Rose and Judd, 2014). Only then will a complete understanding of the species be obtained, as over half its range extends into Mexico.

North American tortoises are known to be keystone species. They influence community structure, including vegetation cover by grazing and seed dispersal, and also provide refuge for other species that use their burrows (Diemer, 1986; Rose and Judd, 2014). The ecological role that the Texas Tortoise has in its ecosystem remains unclear but further studies can help provide a greater insight into this unique and understudied species. This will, in turn, help in its conservation and the conservation of other species it influences.

APPENDIX SECTION

APPENDIX I

TABLES

Table 1: Various datasets of observation points used to build habitat suitability models, based on accuracy in collection of the points, for Texas Tortoise (*Gopherus berlandieri*). The datasets were split into three; points with less than 10 mile accuracy, points with less than 500 meter accuracy and points with less than 10 meter accuracy. The tissue collection at Texas State University and online databases were used to collect this data.

Category	Name of file	Number of points	Source
Points with less than 10 miles accuracy	Total	610	BISON, Dr. Rose, VertNet, iNaturalist, MRJ Forstner Tissue Collection at Texas State University, and points from various observers
Points with less than 500 meter accuracy	<500m	251	iNaturalist, MRJ Forstner Tissue Collection at Texas State University, and points from various observers
Points with less than 10 meter accuracy	<10m	180	MRJ Forstner Tissue Collection at Texas State University

Table 2: Six climatic variables in the form of 30 arc-second cell size ESRI-raster used as environmental layers to build the various habitat suitability models for Texas Tortoise (*Gopherus berlandieri*), using Maxent v3.3.k.

Name of Variable	Raster Name	Units
Mean Diurnal Range (Mean of monthly(maximum temperature-minimum temperature)	Bio2	°C*10
Isothermality (Mean Diurnal Range/Temperature Annual Range)	Bio3	-
Minimum Temperature of Coldest Month	Bio6	°C*10
Mean Temperature of Warmest Quarter	Bio10	°C*10
Precipitation Seasonality (Coefficient of Variation)	Bio15.2	-
Precipitation of Warmest Quarter	Bio18	Millimeters

Table 3: Percentage Shrub Cover, obtained from LANDFIRE, and used as an environmental layer to build the various habitat suitability models for Texas Tortoise (*Gopherus berlandieri*), using Maxent v3.3.k.

LANDFIRE Existing Vegetation Cover Type	**Shrub value**
Shrub Cover >= 10 and < 20%	15
Shrub Cover >= 20 and < 30%	25
Shrub Cover >= 30 and < 40%	35
Shrub Cover >= 40 and < 50%	45
Shrub Cover >= 50 and < 60%	55
Shrub Cover >= 60 and < 70%	65
Shrub Cover >= 70 and < 80%	75
Shrub Cover >= 80 and < 90%	85
Shrub Cover >= 90 and < 100%	95

Table 4: Percentage Herb Cover, obtained from LANDFIRE, and used as an environmental layer to build the various habitat suitability models for Texas Tortoise (*Gopherus berlandieri*), using Maxent v3.3.k.

LANDFIRE Existing Vegetation Cover Type	**Herb value**
Herb Cover >= 10 and < 20%	15
Herb Cover >= 20 and < 30%	25
Herb Cover >= 30 and < 40%	35
Herb Cover >= 40 and < 50%	45
Herb Cover >= 50 and < 60%	55
Herb Cover >= 60 and < 70%	65
Herb Cover >= 70 and < 80%	75
Herb Cover >= 80 and < 90%	85
Herb Cover >= 90 and < 100%	95

Table 5: Percentage Forest Canopy Cover, obtained from LANDFIRE, and used as an environmental layer to build the various habitat suitability models for Texas Tortoise (*Gopherus berlandieri*), using Maxent v3.3.k.

LANDFIRE Existing Vegetation Cover Type	**Forest value**
Forest Cover >= 10 and < 20%	15
Forest Cover >= 20 and < 30%	25
Forest Cover >= 30 and < 40%	35
Forest Cover >= 40 and < 50%	45
Forest Cover >= 50 and < 60%	55
Forest Cover >= 60 and < 70%	65
Forest Cover >= 70 and < 80%	75
Forest Cover >= 80 and < 90%	85
Forest Cover >= 90 and < 100%	95

Table 6: Summary of models for the assessment of potential habitat model parameters for the Texas Tortoise (*Gopherus berlandieri*) built using Maxent v3.3.k. Six different models were built based on datasets with different levels of accuracy of samples (Table 1) as well as different variables used as predictors in model building. The number of training points, test points, AUC and standard deviation are included for each model built.

Model	Variables included	Number of Training Points	Number of Test Points	Average Test AUC for replicates	Standard Deviation
1. Model with 180 points and 10 variables	Bio6, bio15.2, ksat2, Percsand_fin, nlcd, for, shrub, ag, dev_2_cat, herb.	144	36	0.966	0.008
2. Model with 251 points and 10 variables	Bio6, bio15.2, ksat2, Percsand_fin, nlcd, for, shrub, ag, dev_2_cat, herb.	201	50	0.949	0.004
3. Model with 610 points and 10 variables	Bio6, bio15.2, ksat2, Percsand_fin, nlcd, for, shrub, ag, dev_2_cat, herb.	490	120	0.926	0.007
4. Model with 180 points and 9 variables	Bio6, bio15.2, ksat2, Percsand_fin, nlcd, for, shrub, ag, herb.	144	36	0.976	0.005
5. Model with 251 points and 9 variables	Bio6, bio15.2, ksat2, Percsand_fin, nlcd, for, shrub, ag, herb.	201	50	0.954	0.006
6. Model with 610 points and 9 variables	Bio6, bio15.2, ksat2, Percsand_fin, nlcd, for, shrub, ag, herb.	490	120	0.929	0.008

Table 7: Summary of AIC_C, AIC, and BIC values for the various models, built for Texas Tortoise (*Gopherus berlandieri*) using Maxent v3.3.k, calculated using ENMTools (Warren *et al.,* 2010). Six different models were built and their corresponding output using ENMTools are as below. AIC_C for model one was the lowest (5146.5) and was used for subsequent analysis.

Model	Log Likelihood	Parameters	AIC score	AICc score	BIC score	ΔAICc
1. 180 points+10 variables	-2481	56	5074	5146.5	5241	0
2. 251 points+10 variables	-3664	56	7440	7482	7628	2335.5
3. 610 points+10 variables	-6289	66	12709	12741	12964	7594.5
4. 180 points+9 variables	-2560	55	5229	5298	5393	151.5
5. 251 points+9 variables	-3753	52	7610	7645	7784	2498.5
6. 610 points+9 variables	-6477	72	13097.5	13135	13375	7988.5

Table 8: Microsatellite loci that were cross amplified from Gopher Tortoise (*Gopherus polyphemus*), in Texas Tortoise (*Gopherus berlandieri*) (n=55). All four were previously unidentified in Texas Tortoise. The universal M13 forward and reverse pig tails that were used along with the various loci are also added to the primer sequences. For each locus, T_a is the annealing temperature, A_R is the allelic richness, H_0 is the observed heterozygosity and H_A is the expected heterozygosity.

Locus	Primer sequence (5'-3')	T_a (°C)	Size Range (bp)	A_R	H_0	H_E	Allelic Size Range
GopoA007	F:TGTAAAACGACGGCCAGTGGAAATGGATGGCTGAAG R: GTTTCTTCGTCTTATTGTTGACCAGATG	52°	293-302	2	0.47170	0.44654	9
GopoA008	F:TGTAAAACGACGGCCAGTACTTGCATAGGGGTAAAACTTG R: GTTTCTTCTGCTACCAGTCCGTTCTTAA	54°	283-316	10	0.67273	0.74028	33
GopoA012	F:TGTAAAACGACGGCCAGTATCTGGCTTAGAGGTGGAAACT R: GTTTCTTAATAGGTCCTGATCCATGACTG	54°	286-292	3	0.60377	0.59030	6
GopoA111	F:TGTAAAACGACGGCCAGTTAACCCACCTTGGAAGTAATC R: GTTTCTTATGGCTCAGCAACACATTT	54°	197-200	2	0.10909	0.16681	3
Mean	-	-	-	4.25	0.46432	0.48598	12.75

Table 9: Twenty-nine microsatellite loci tested for amplification in Texas Tortoise (*Gopherus berlandieri*), based on microsatellite loci developed by Kreiser *et al*., 2013. Results from preliminary Polymerase Chain Reactions are also indicated. Y=Yes (amplification), N=No (amplification).

Microsatellite Loci	Amplified during PCR	Previously tested by Kreiser *et al.*, 2013
GopoA003	Y	Y
GopoA006	Y	Y
GopoA007	Y	N
GopoA008	Y	N
GopoA009	Y	Y
GopoA012	Y	N
GopoA106	Y	Y
GopoA110	N	Y
GopoA111	Y	N
GopoA117	N	N
GopoA122	Y	N
GopoB004	N	Y
GopoB011	Y	Y
GopoB012	Y	Y
GopoB102	Y	Y
GopoB103	Y	Y
GopoB104	Y	Y
GopoB112	N	Y
GopoB118	Y	Y
GopoB120	Y	Y
GopoC001	Y	Y
GopoD004	Y	Y
GopoD006	Y	Y
GopoD007	Y	Y
GopoD011	Y	Y
GopoD102	N	N
GopoD107	Y	Y
GopoD126	Y	Y
GopoD128	Y	Y

APPENDIX I
FIGURES

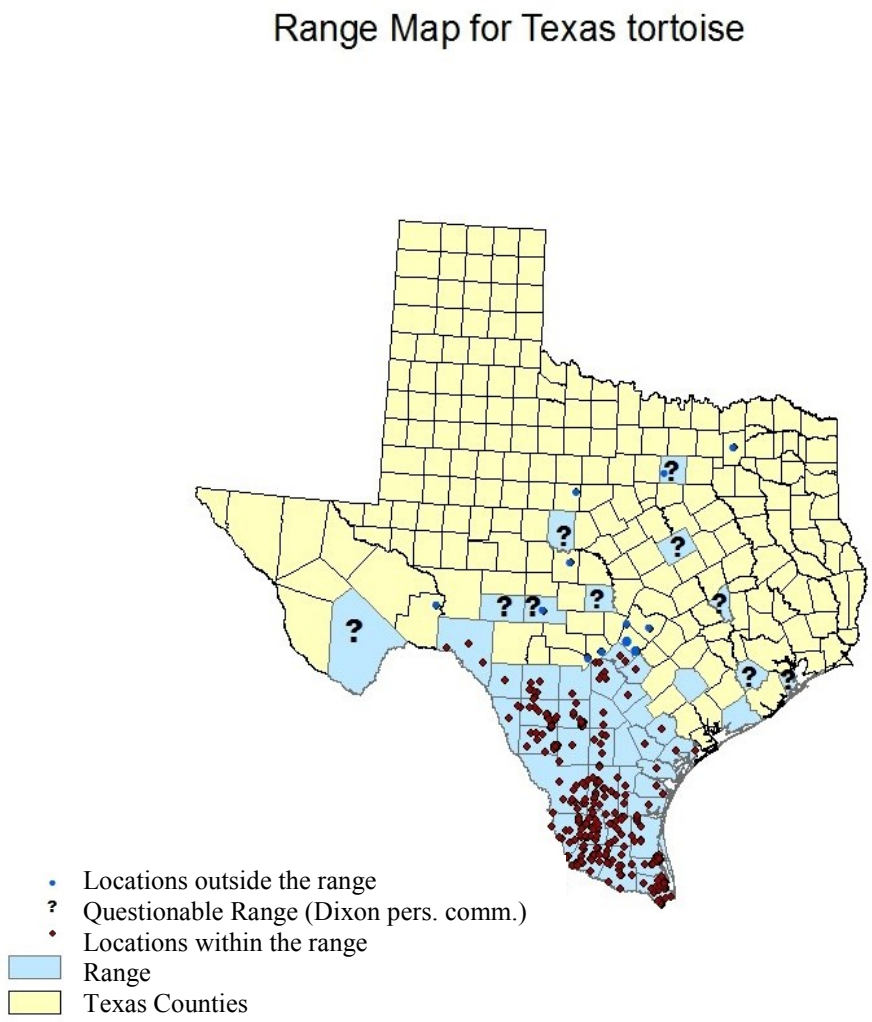
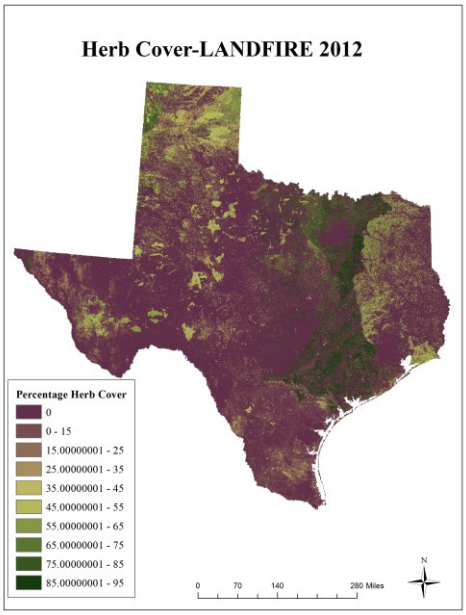
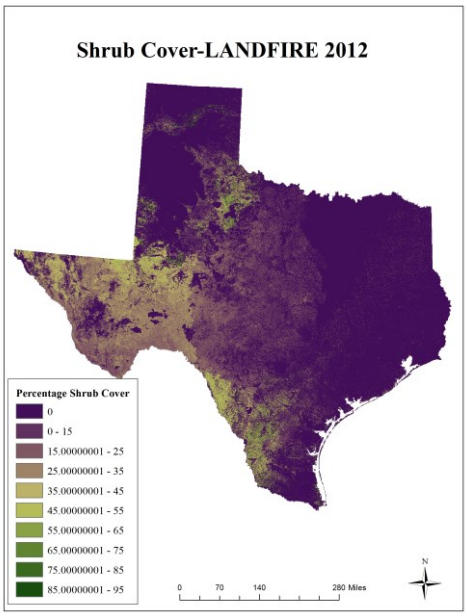
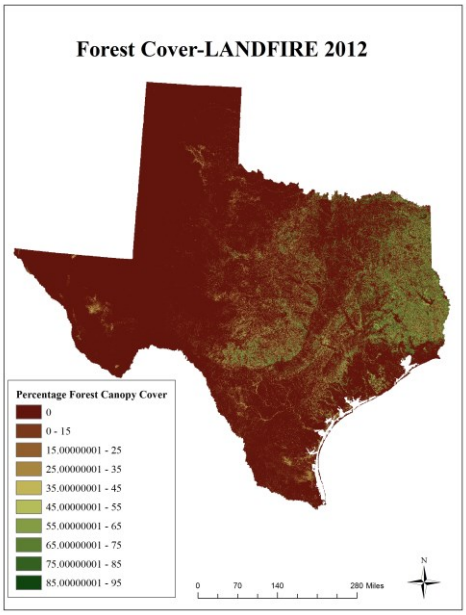
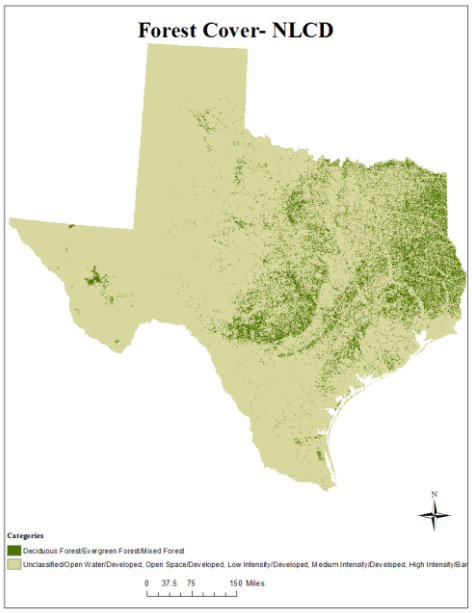


Figure 1: Texas map depicting the historic and current range for Texas Tortoise (*Gopherus berlandieri*) with locations within the range and outside. Locations outside might indicate potential relocations or pet tortoises released into the wild. Locations with question marks indicate parts of the range that might be questionable according to Dixon (2013).

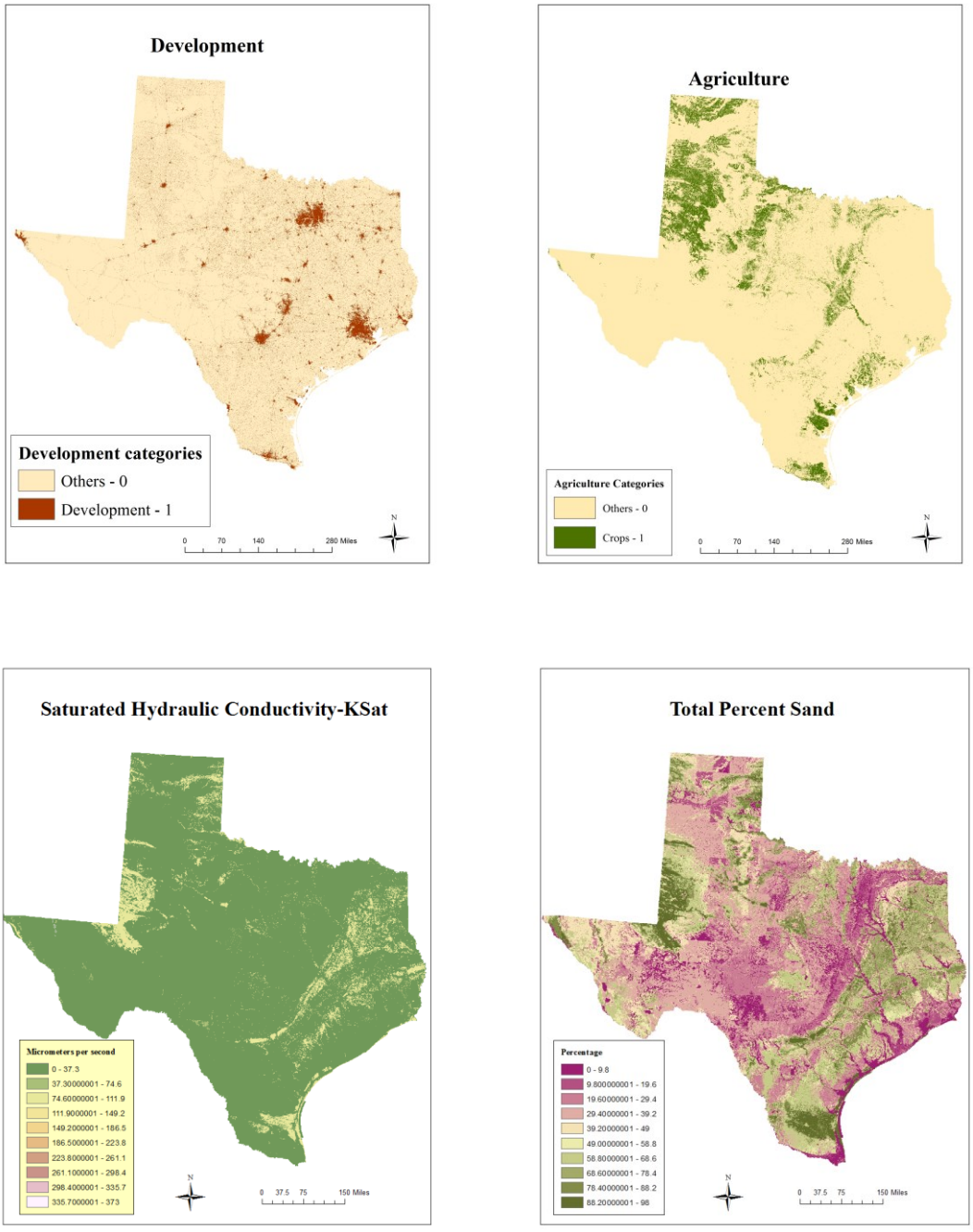




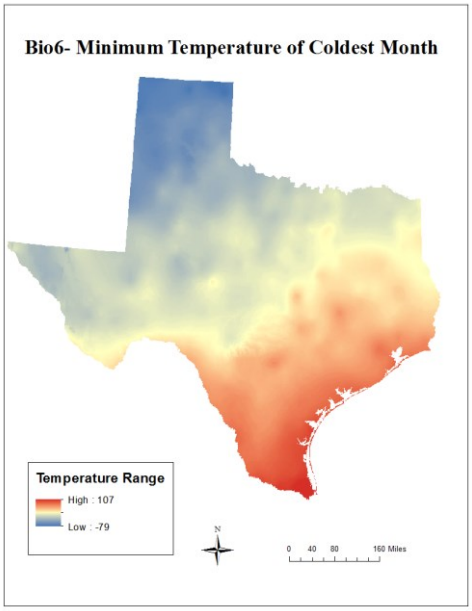


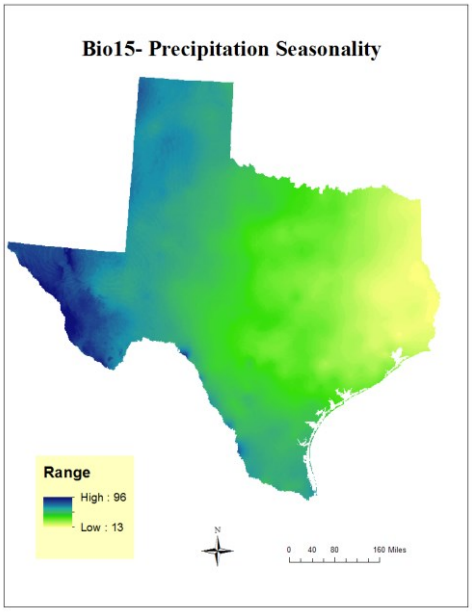


Figures 2a-2d: Final Land Cover variables (Forest Cover-both NLCD and LANDFIRE, Shrub Cover, and Herb Cover) used as environmental layers in Maxent to build habitat suitability models for Texas Tortoise (*Gopherus berlandieri*).



Figures 3a-3d: Final Land Cover variables (Development and Agriculture) and Soil variables (KSat and Total Percent Sand) used as environmental layers in Maxent, to build habitat suitability models for Texas Tortoise (*Gopherus berlandieri*).





Figures 4a-4b: Final Climatic layers (Minimum Temperature of Coldest Month and Precipitation Seasonality) used as environmental layers in Maxent to build habitat suitability models for Texas Tortoise (*Gopherus berlandieri*).

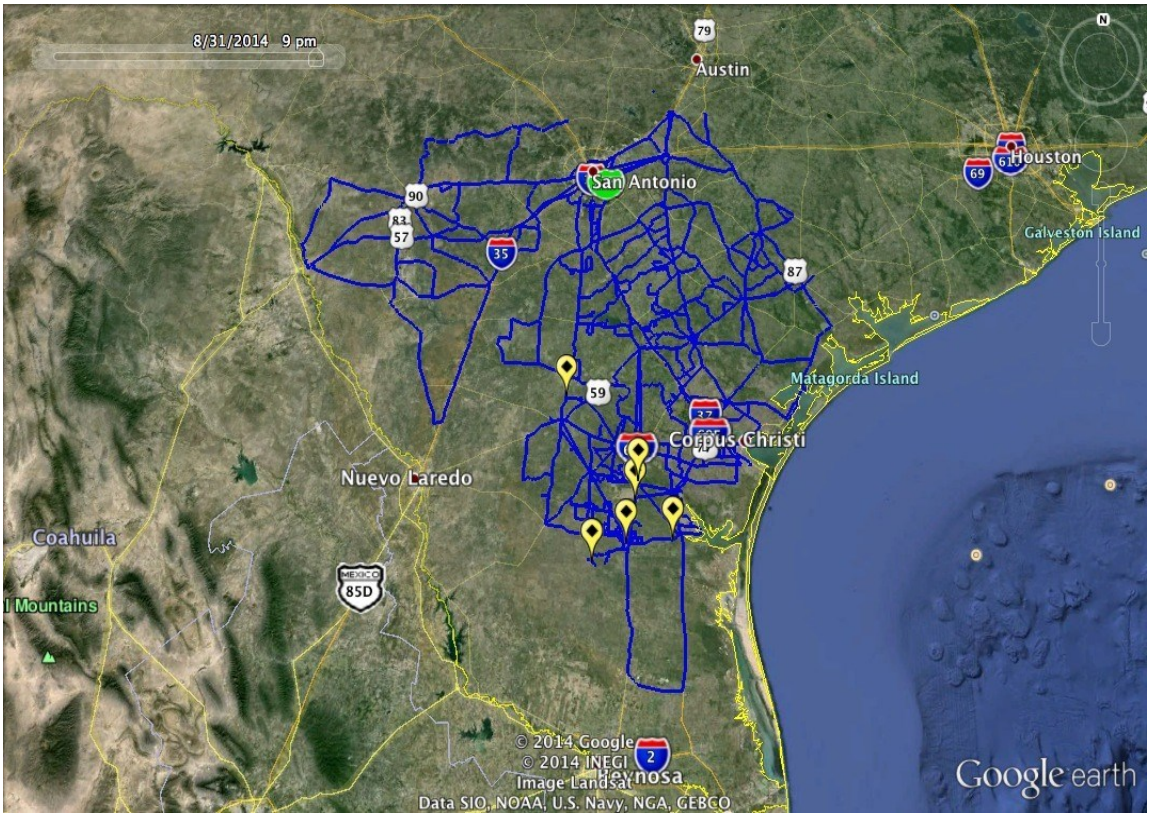


Figure 5: Texas Tortoise (*Gopherus berlandieri*) road survey routes conducted from March-October 2014 in Texas. Routes are displayed in blue and tortoises found during the surveys are depicted by yellow markers.

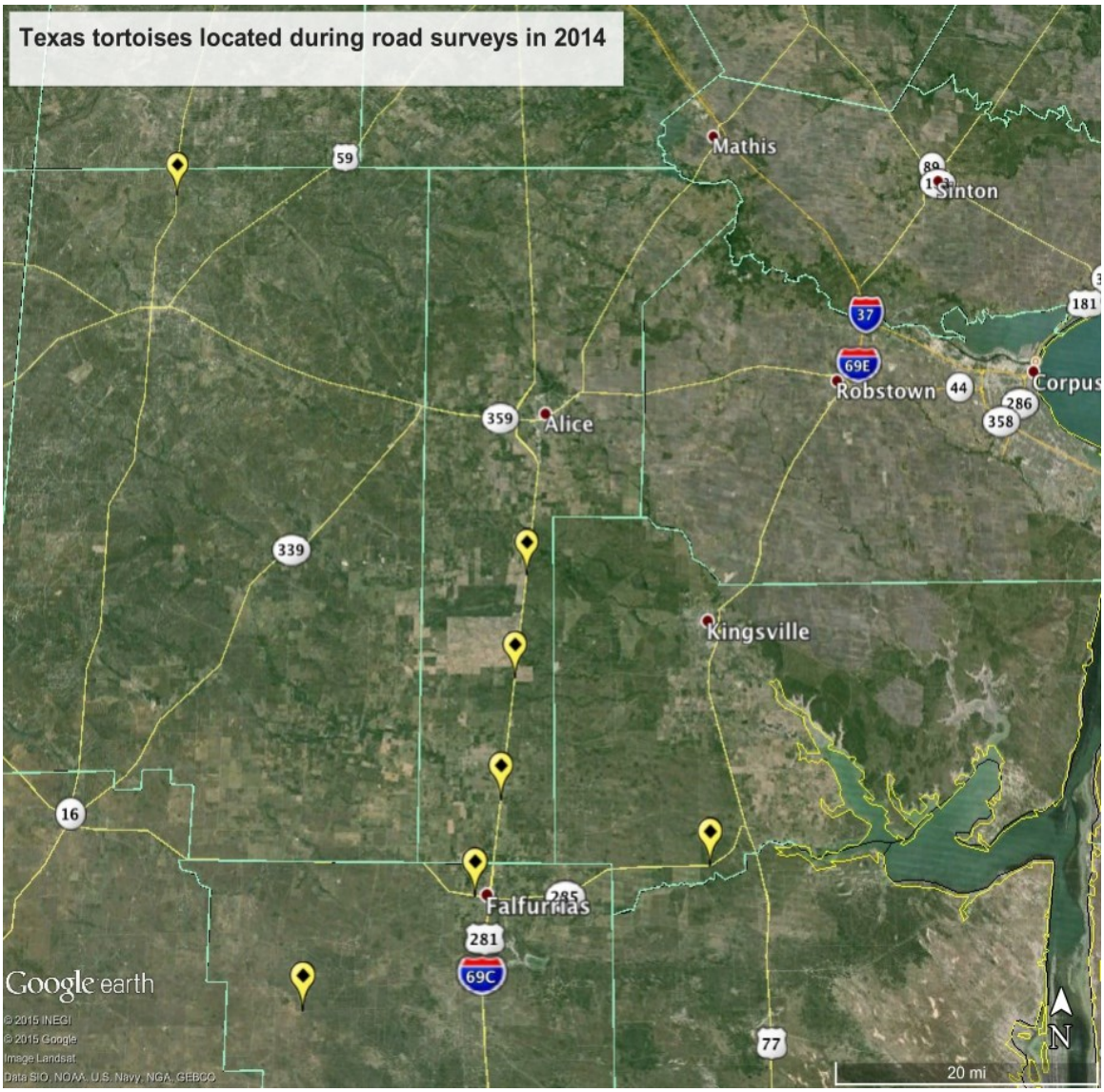


Figure 6: Texas Tortoises (*Gopherus berlandieri*) found during road surveys conducted from March-October 2014. All tortoises were found east of Highway 16 and around or just north of Highway 285, as indicated by the yellow markers in the figure.

Figure 7: Texas Tortoise (*Gopherus berlandieri*) found in a park in Comal County in 2014. Markings and body condition indicate that the tortoise was a pet.

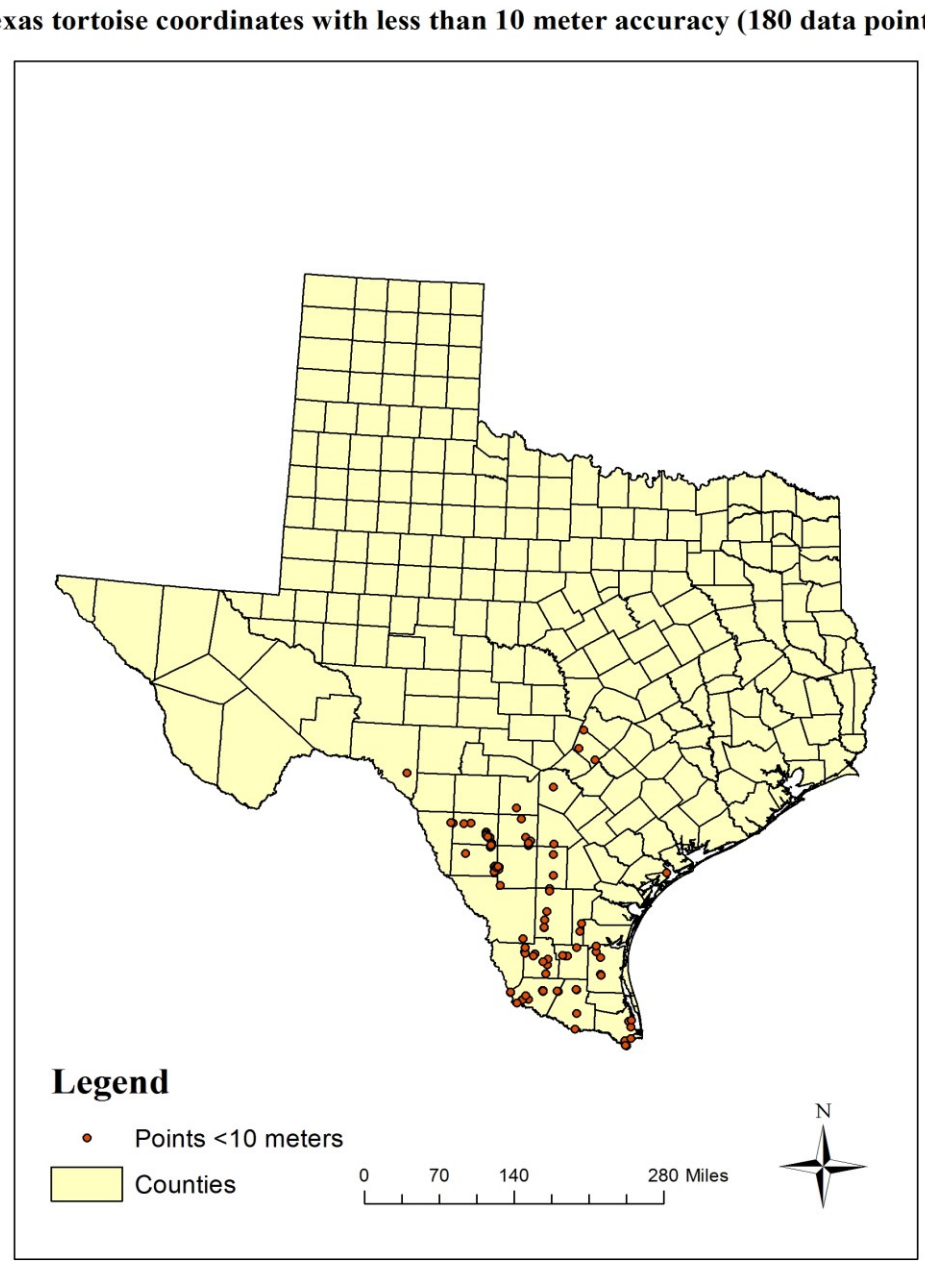


Figure 8: Map depicting the first model built, in Maxent for Texas Tortoise (*Gopherus berlandieri*), with 180 coordinates that had less than ten meter accuracy. The points were obtained from the MRJ Forstner Tissue Collection at Texas State University.

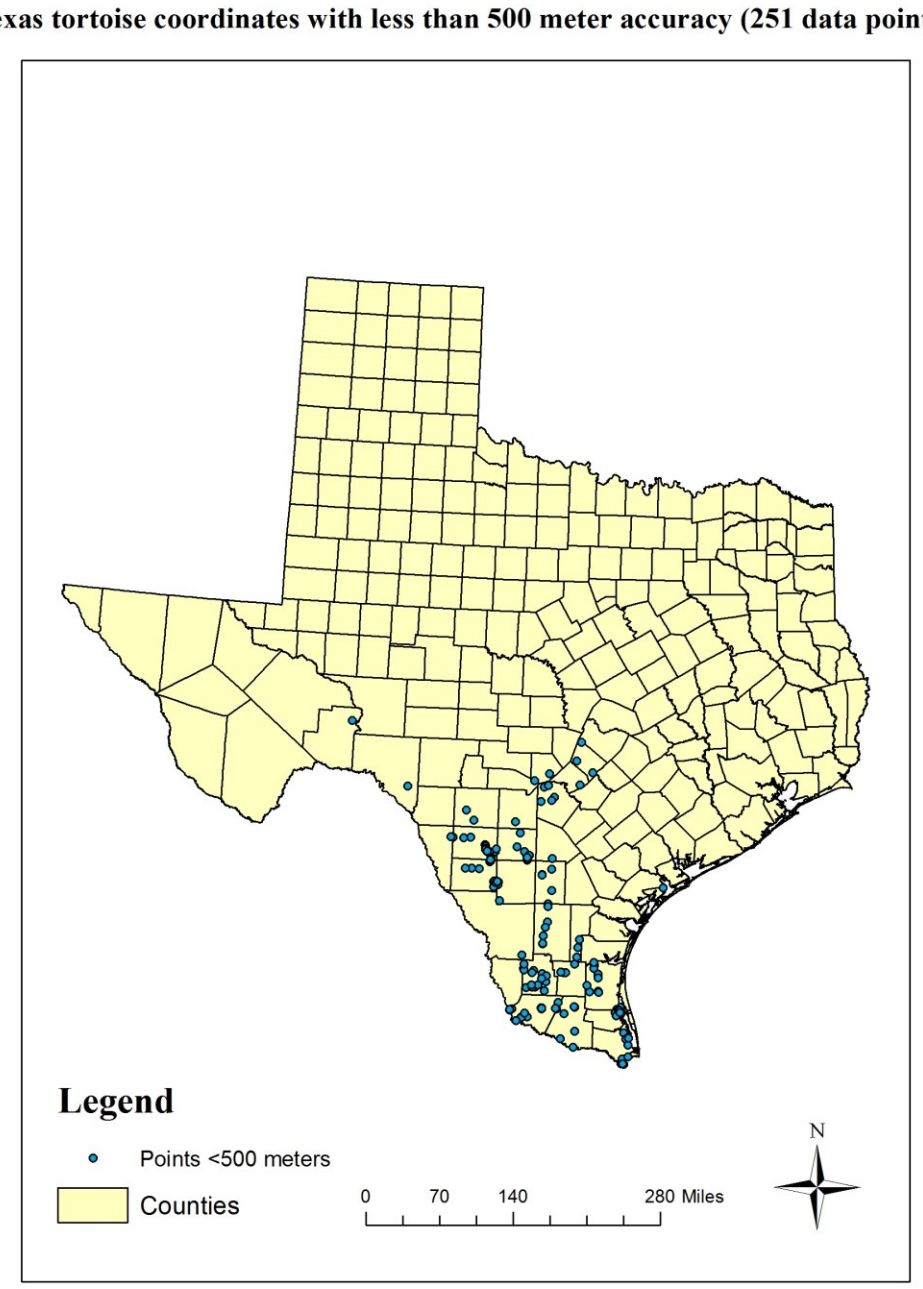


Figure 9: Map depicting the second model built, in Maxent for Texas Tortoise (*Gopherus berlandieri*), with 251 coordinates that had less than 500 meter accuracy. The points were obtained from the MRJ Forstner Tissue Collection at Texas State University and various online databases.

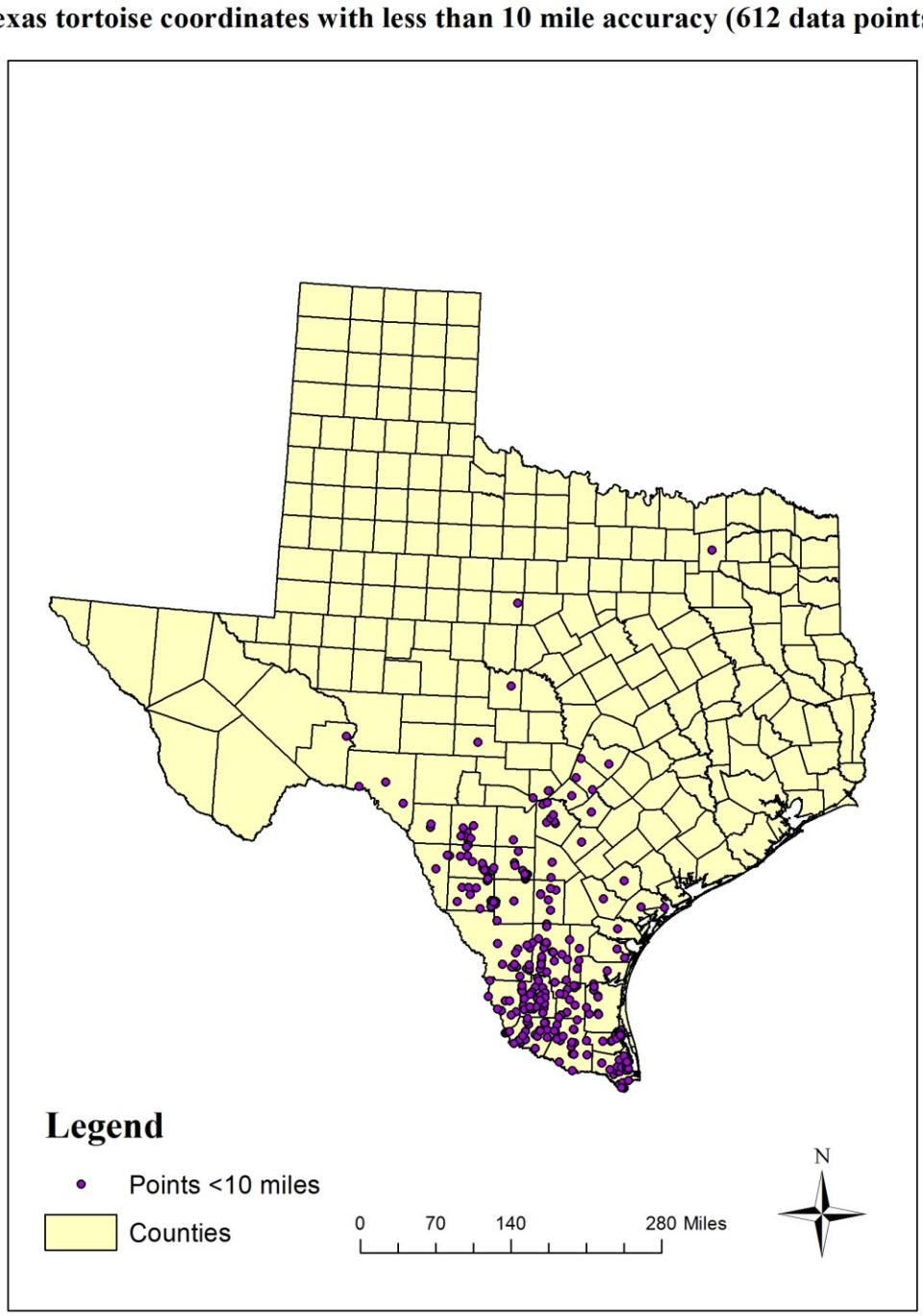


Figure 10: Map depicting the third model built, in Maxent for Texas Tortoise (*Gopherus berlandieri*), with 610 coordinates that had less than a ten mile accuracy. The points were obtained from the MRJ Forstner Tissue Collection at Texas State University and various online databases.

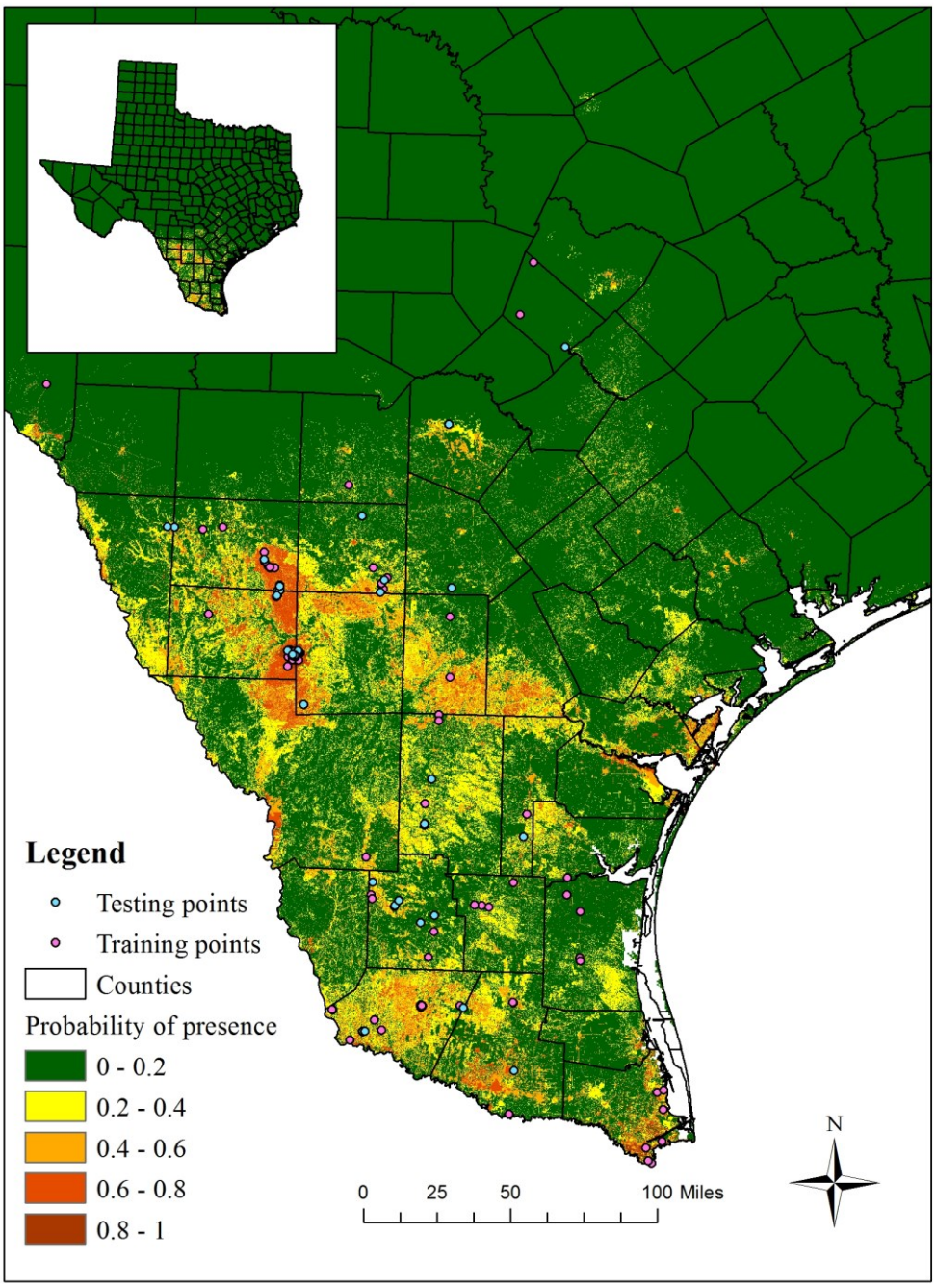


Figure 11: Map of Texas depicting areas of probable presence or suitability for Texas Tortoise (*Gopherus berlandieri*) based on model one built in Maxent. 180 samples were used to build this model, with 36 points for testing (blue) and 144 points for training (pink).

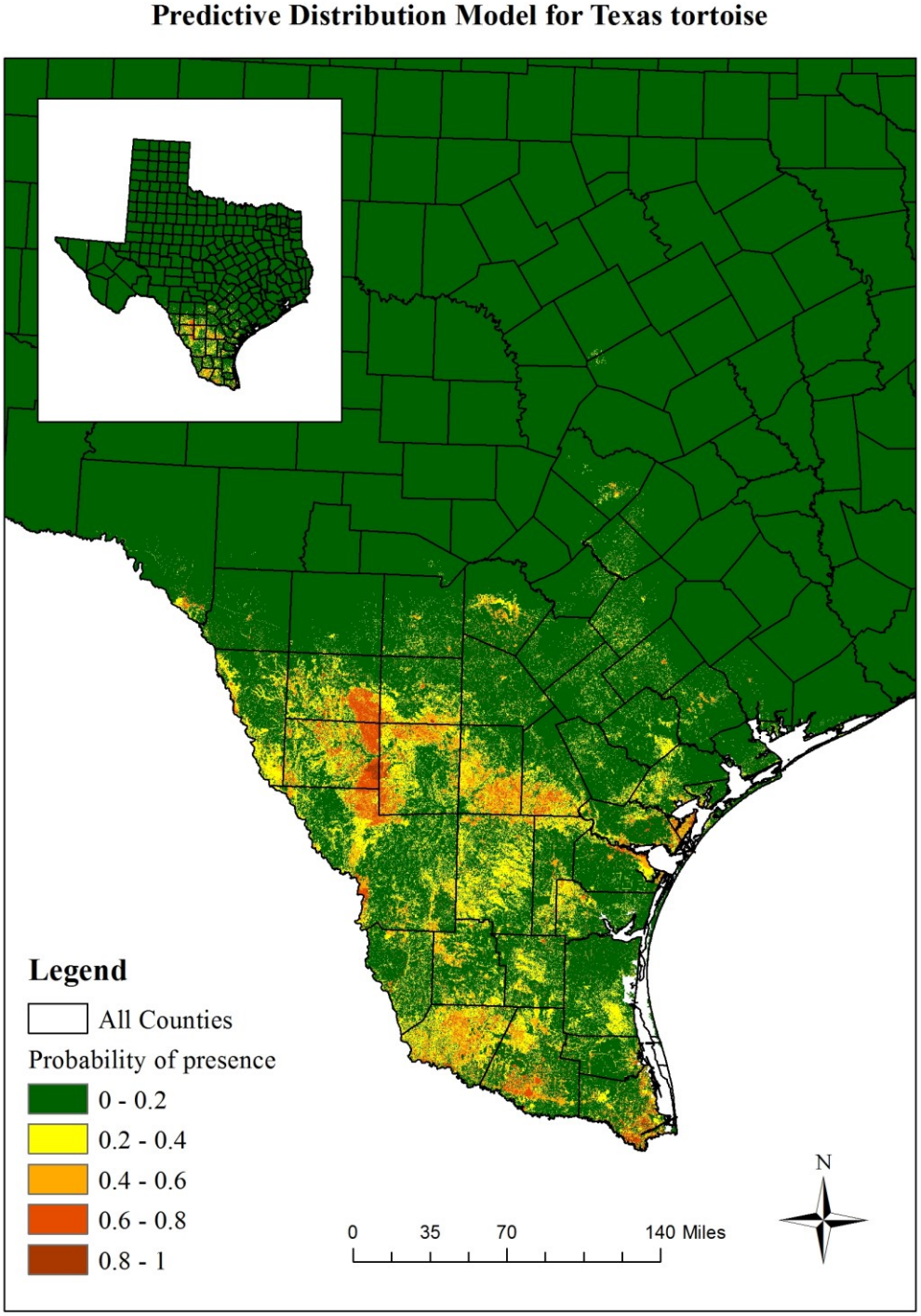


Figure 12: Map of Texas depicting areas of probable presence or suitability for Texas Tortoise, (*Gopherus berlandieri*) based on model one built in Maxent. 180 samples were used to build this model, with 36 points for testing and 144 points for training. Areas of high suitability are in red/orange and can be seen in the south most parts of Texas as well as in some areas further north and towards the west. The eastern region has patches of suitability. Suitable areas are also present outside the range of the species.

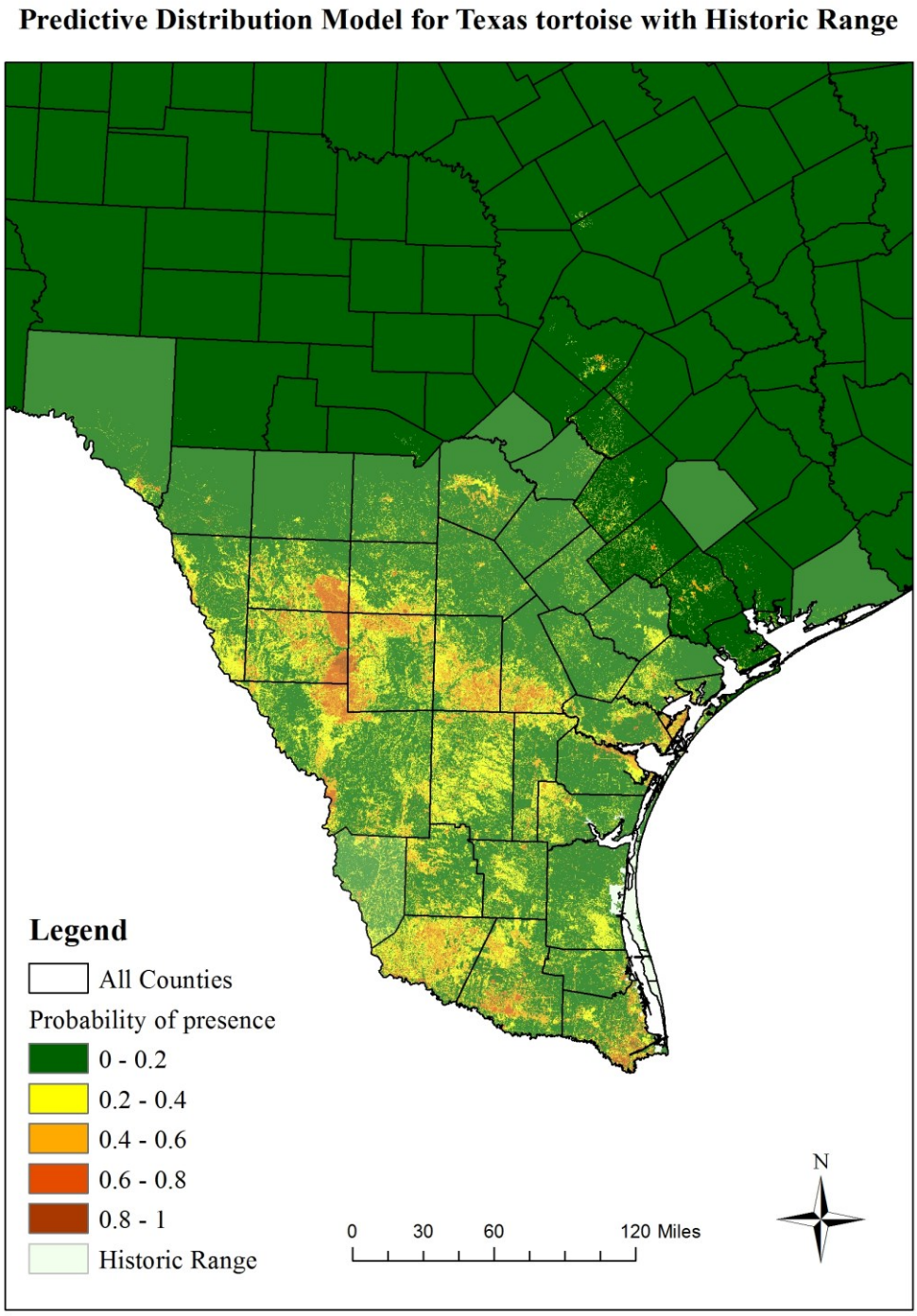


Figure 13: Map of habitat suitability with the Texas Tortoise (*Gopherus berlandieri*) historical range overlaid. Areas of suitability outside this range are visible. Areas in the eastern region are not as well supported as previously thought.

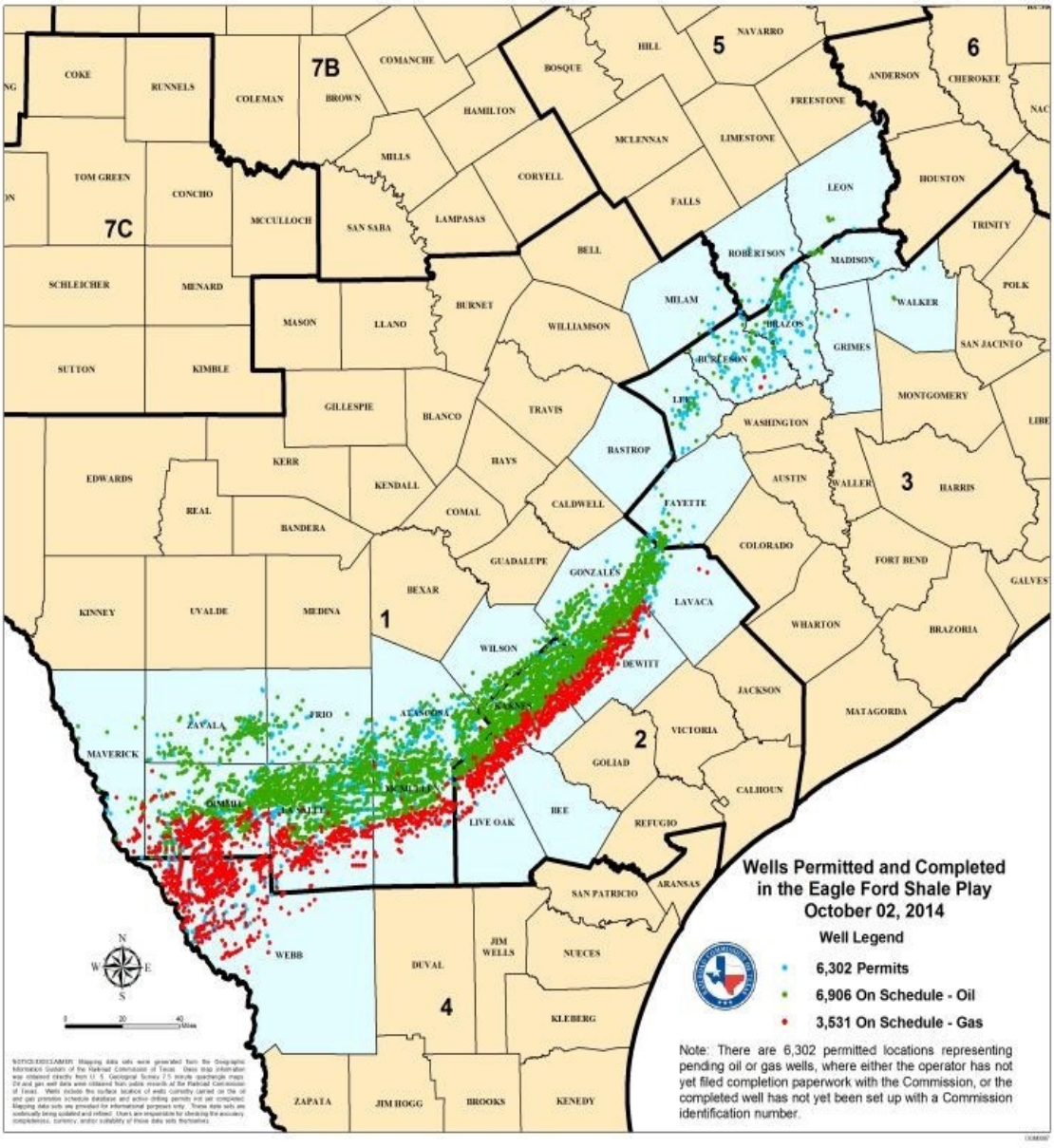


Figure 14: Map of the Eagle Ford Shale hydraulic fracturing wells permitted and completed as of October 2, 2014. Map acquired from the Railroad Commission of Texas Eagle Ford Shale Information webpage (http://www.rrc.state.tx.us/oil-gas/major-oil-gas-formations/eagle-ford- shale/).

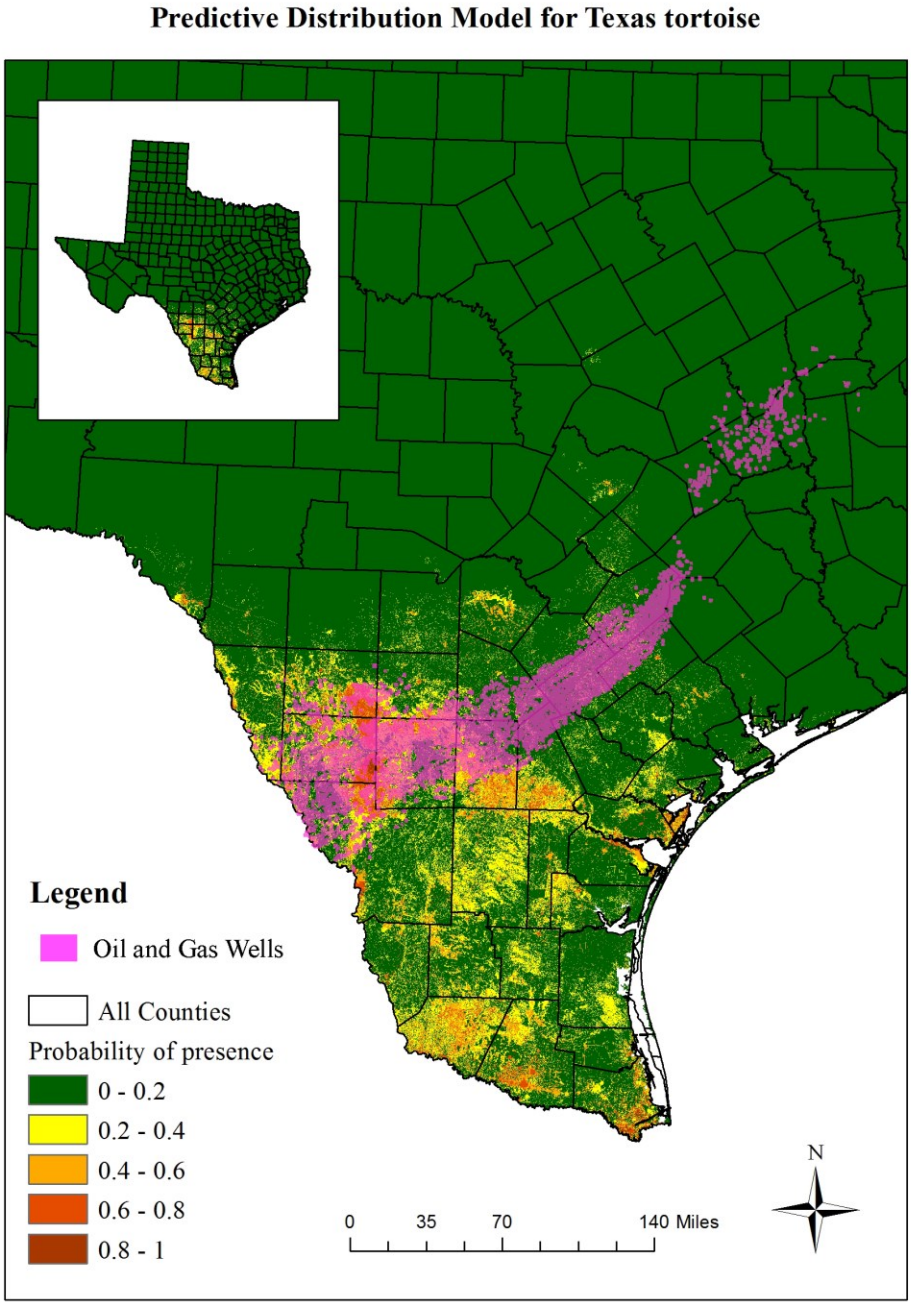


Figure 15: Hybrid map of Texas depicting areas of probable presence or suitability for Texas Tortoise based on selected model one with Eagle Ford Shale hydraulic fracturing wells permitted and completed as of October 2, 2014, superimposed.

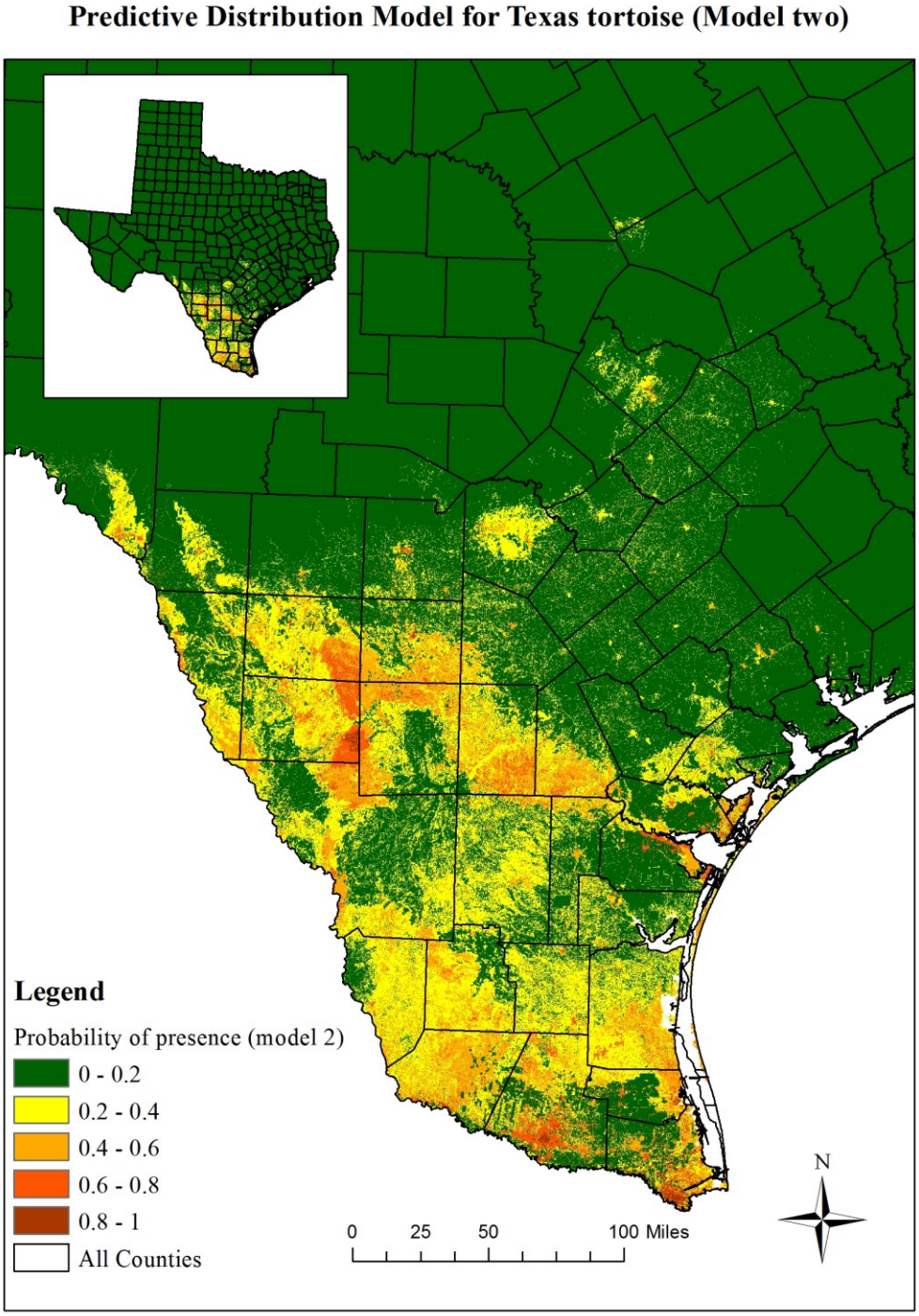


Figure 16: Map of Texas depicting areas of probable presence or suitability for Texas Tortoise, (*Gopherus berlandieri*) based on model two built in Maxent. 251 samples were used for modeling. Areas of high suitability are in red/orange and can be seen in the south most parts of Texas as well as in some areas further north and towards the west. The eastern region has patches of suitability. Suitable areas are also present outside the range of the species.

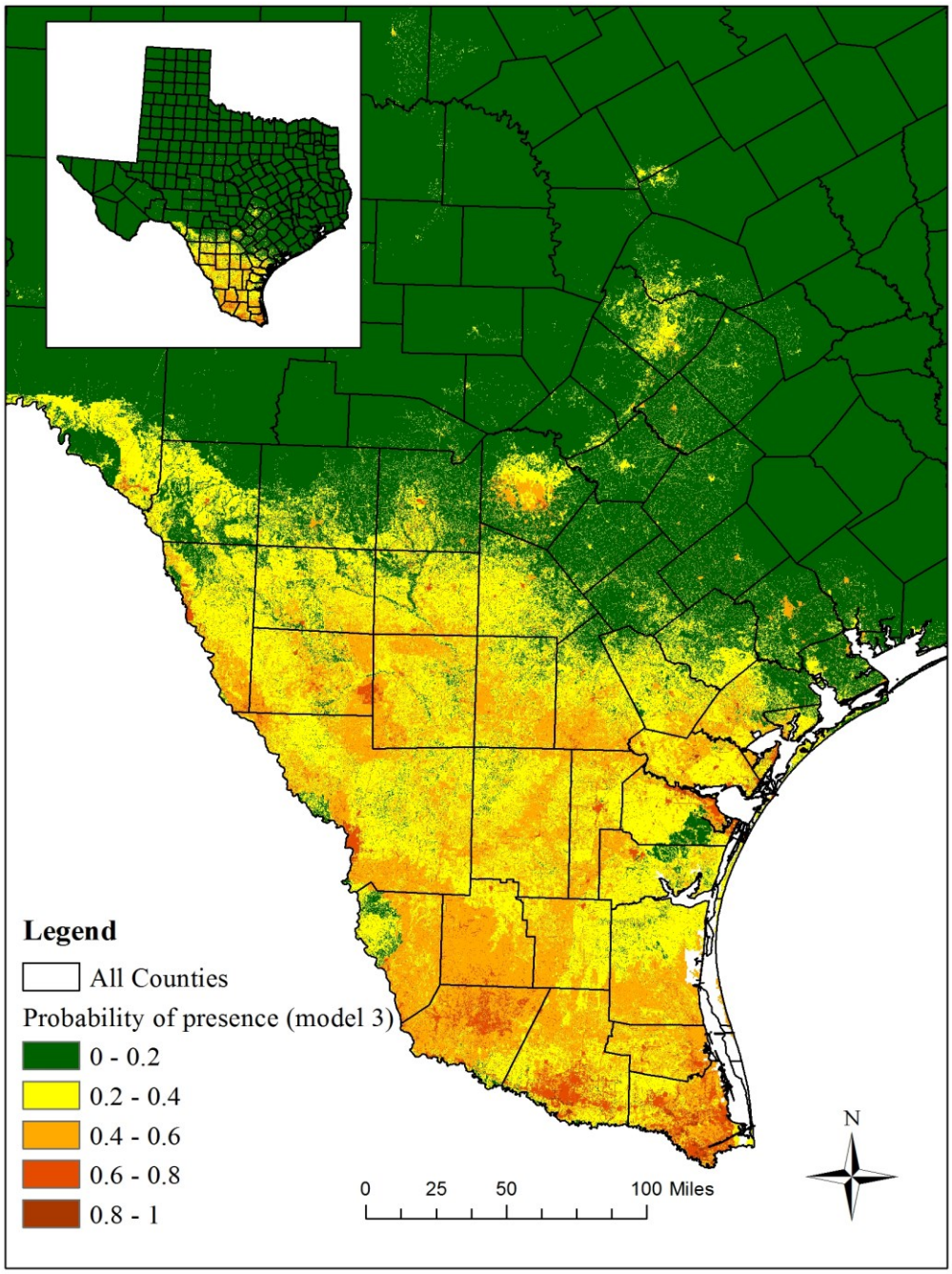


Figure 17: Map of Texas depicting areas of probable presence or suitability for Texas Tortoise, (*Gopherus berlandieri*) based on model three built in Maxent. 610 samples were used for modeling. Areas of high suitability are in red/orange and can be seen in the south most parts of Texas as well as in some areas further north and towards the west. The eastern region has areas of suitability as well. Suitable areas are also present outside the range of the species.

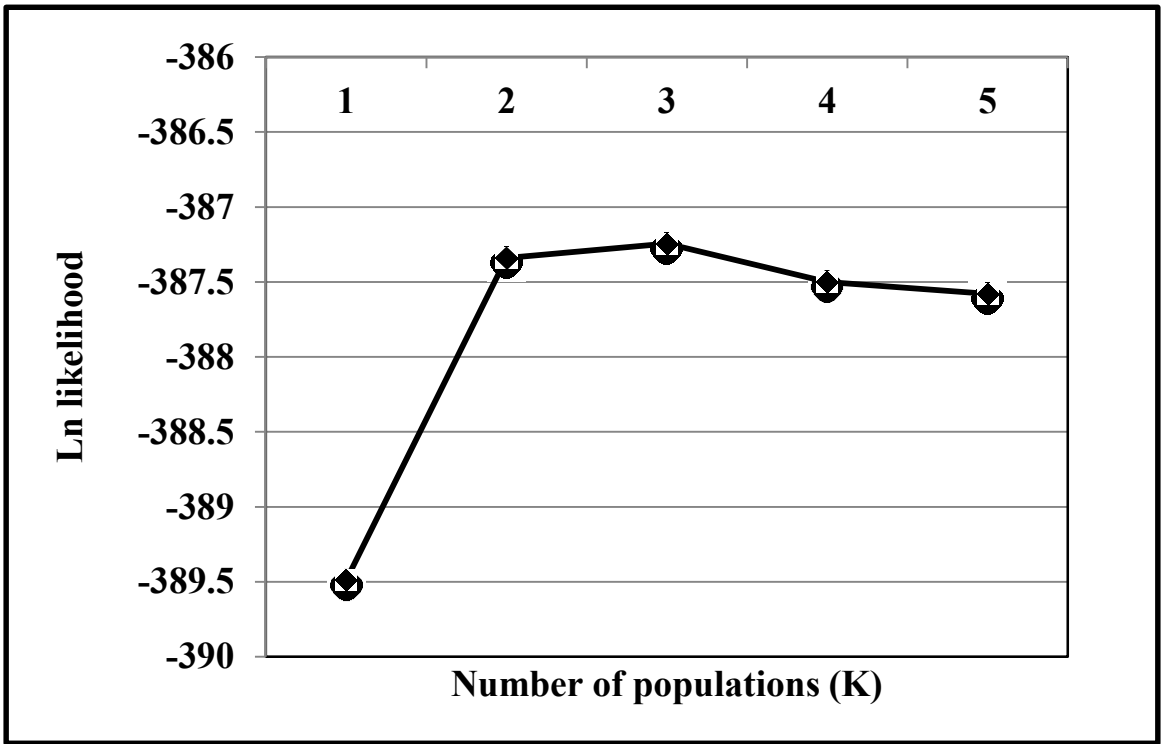


Figure 18: The mean log likelihood of the individual assignment tests at each population (K) tested by STRUCTURE 2.3.4 (Pritchard *et al.*, 2000), using 55 samples that were genotyped for Texas Tortoise (*Gopherus berlandieri*). K of one to five was tested 20 times by STRUCTURE. The highest mean log likehood was examined to approximate number of populations in *G. berlandieri* and was found at K = 2 (Ln = -387.34).

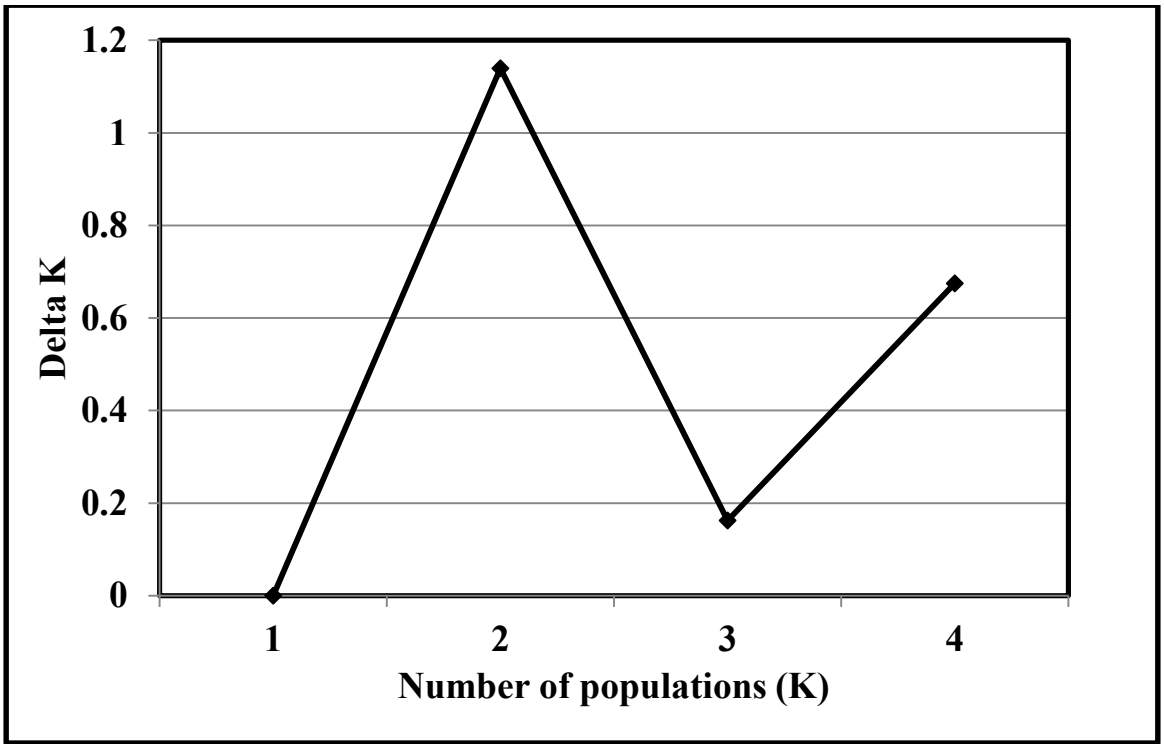


Figure 19: Delta K (rate of change of log likelihood of data) calculated using STRUCTURE Harvester (Evanno *et al*., 2005; Earl and vonHoldt, 2012) to determine number of populations for Texas Tortoise (*Gopherus berlandieri*). The highest Delta K is observed at a K of 2, however Delta K for K of 1 can not be calculated.

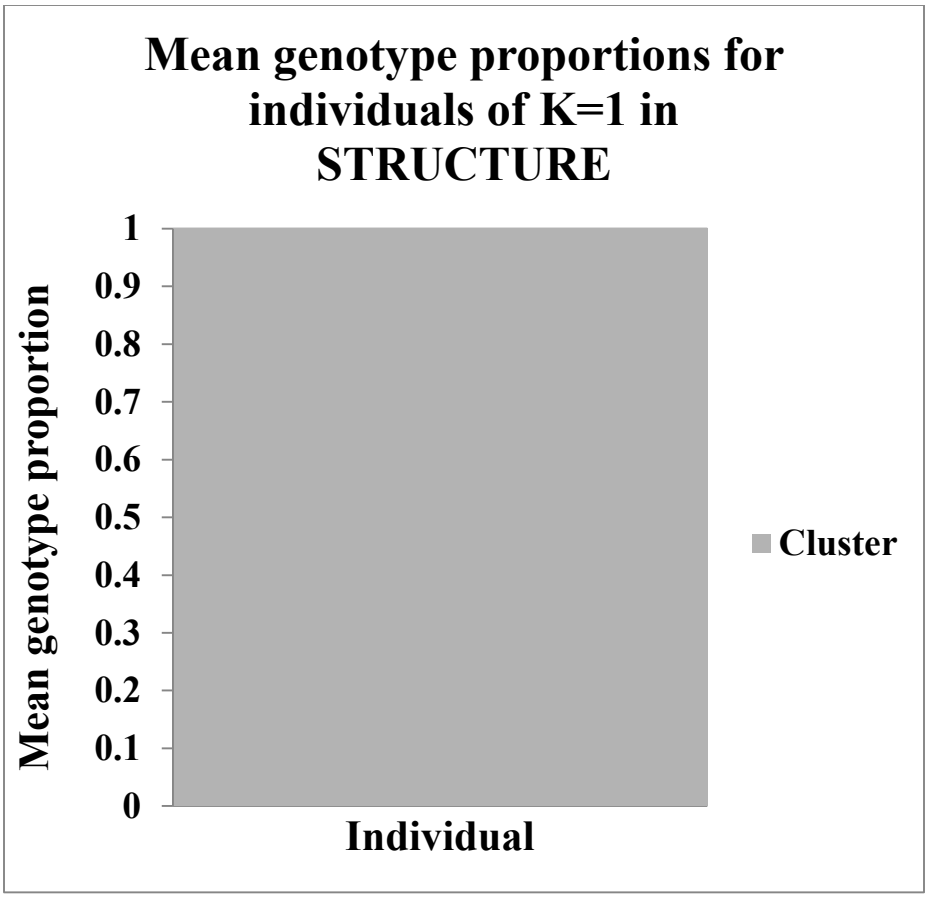


Figure 20: The mean genotype proportion for each individual assigned into one population by STRUCTURE 2.3.4 (Pritchard *et al.*, 2000), using 55 samples that were genotyped for Texas Tortoise (*Gopherus berlandieri*). All individuals showed a mean genotype proportion of 1 for K =1, indicating that *G. berlandieri* has one population.

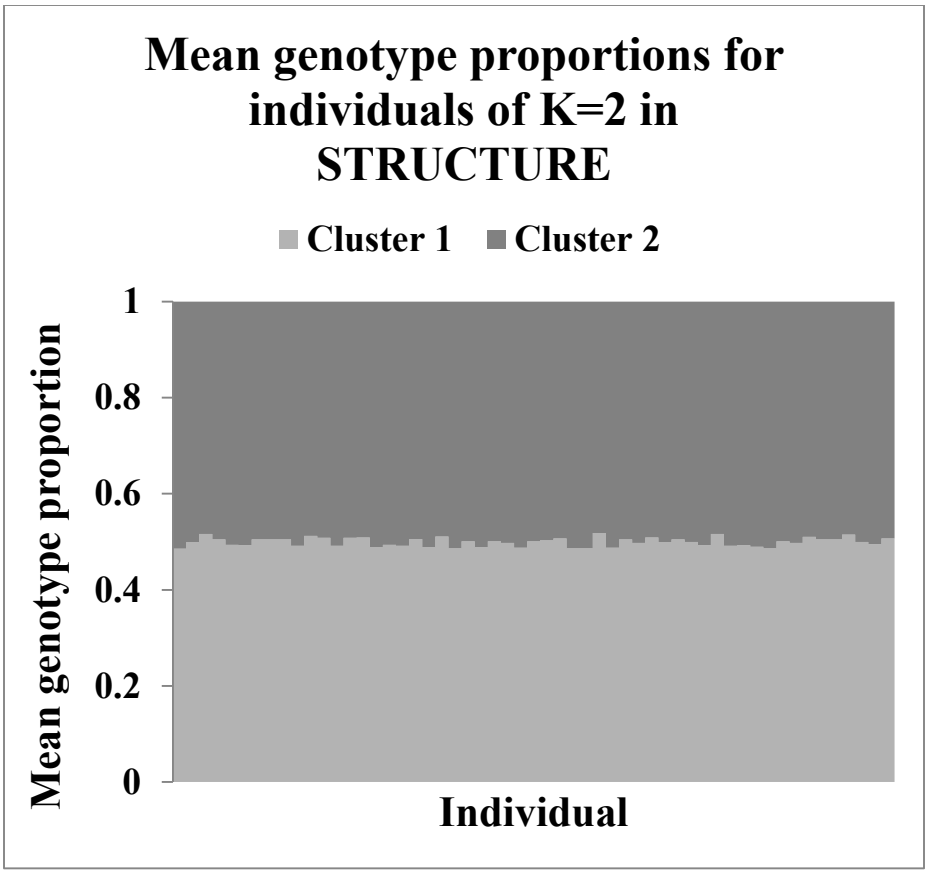


Figure 21: The mean genotype proportion for each individual assigned into two population by STRUCTURE 2.3.4 (Pritchard *et al.*, 2000), using 55 samples that were genotyped for Texas Tortoise (*Gopherus berlandieri*). Dark colored bars indicate the mean genotype proportions of individuals in cluster two and lighter bars indicated individuals in cluster one. All individuals showed an equal mean genotype proportion in both clusters, indicating that *G. berlandieri* has one population.

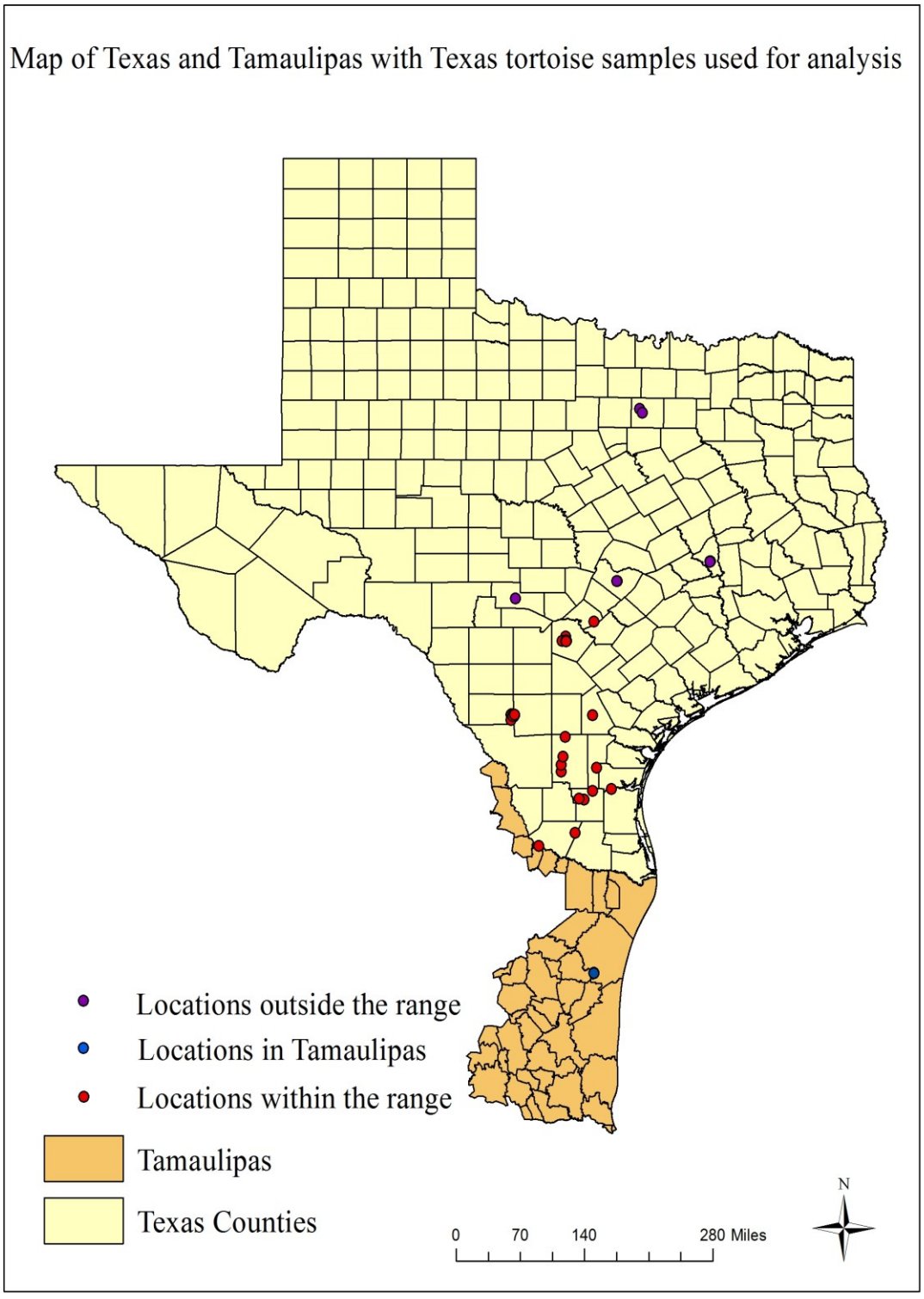
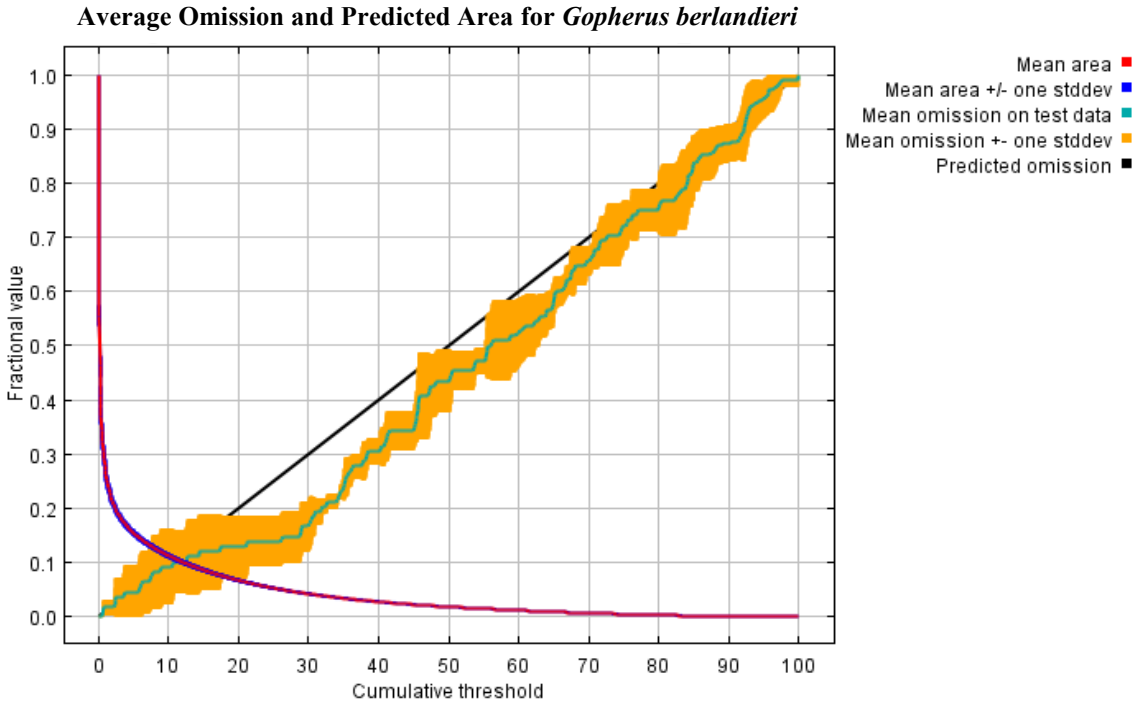


Figure 22: Samples (n = 55) of Texas Tortoise (*Gopherus berlandieri*) used for microsatellite analyses. Samples from within the range in Texas are indicated by red markers, samples from outside the range in Texas are indicated by purple markers, and samples from Tamaulipas (n = 1) are in blue.

APPENDIX II – MAXENT OUTPUT

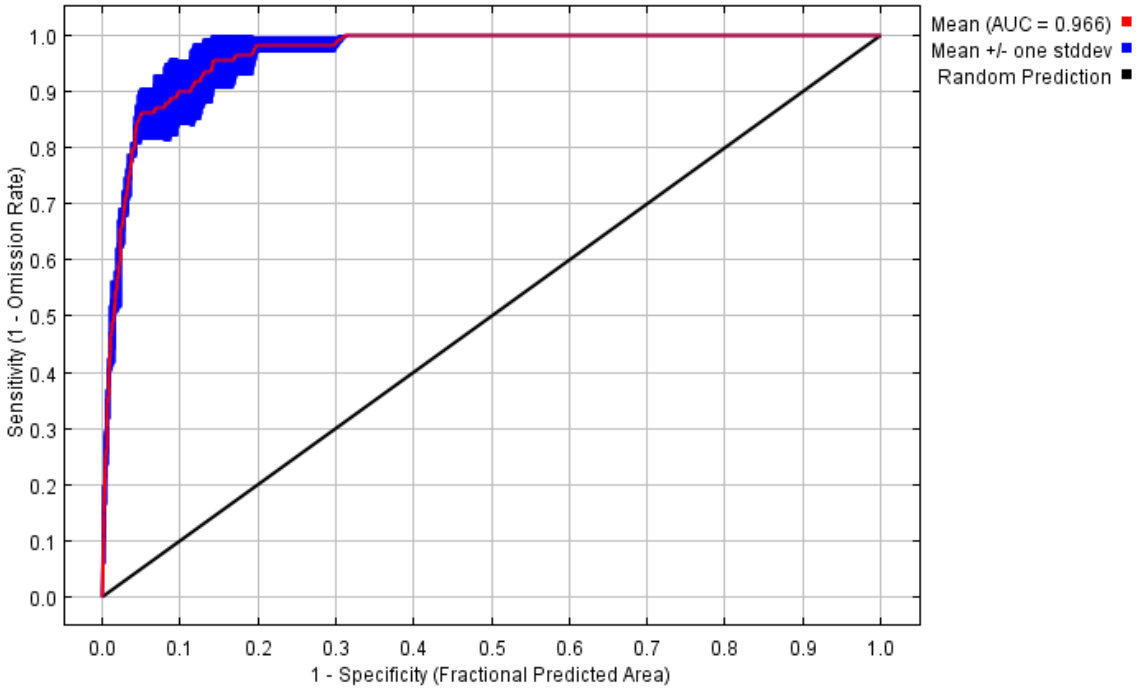
Analysis of omission/commission

The following picture shows the test omission rate and predicted area as a function of the cumulative threshold, averaged over the replicate runs. The omission rate should be close to the predicted omission, because of the definition of the cumulative threshold.

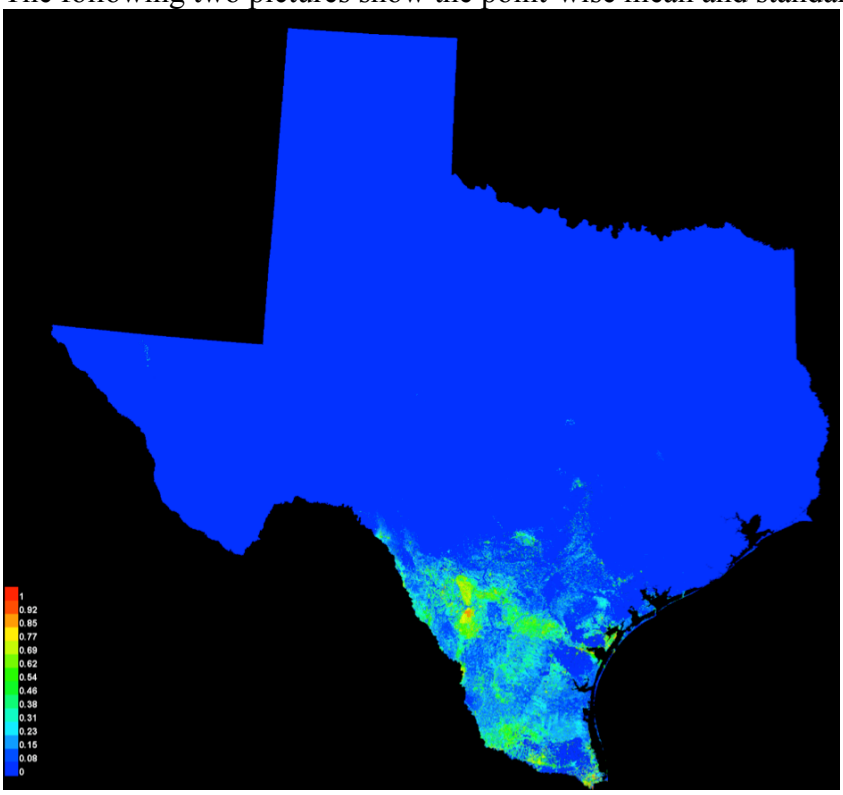


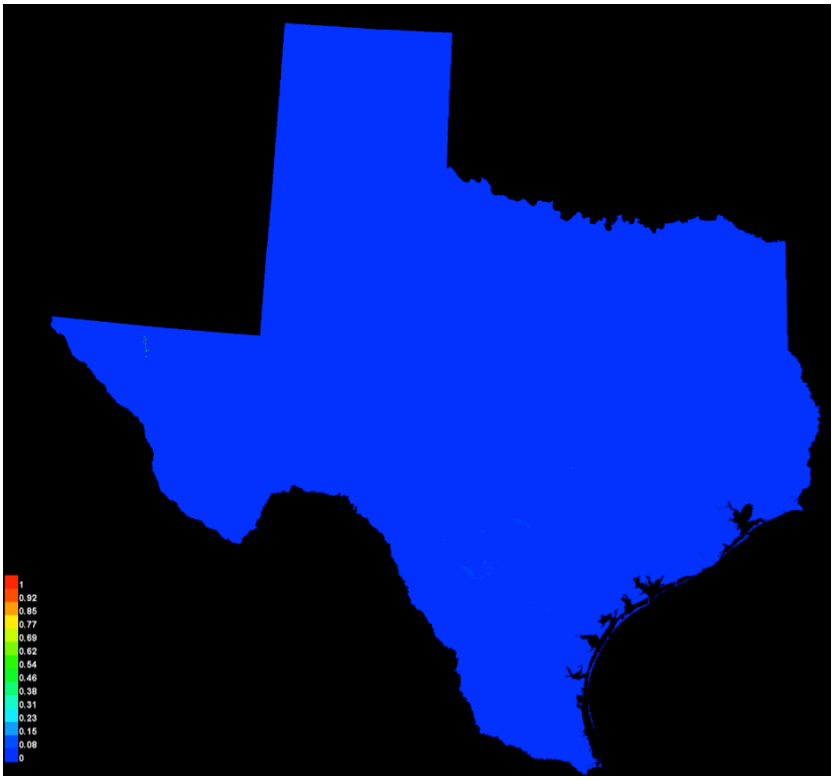
The next graph is the receiver operating characteristic (ROC) curve for the same data, again averaged over the replicate runs. Note that the specificity is defined using predicted area, rather than true commission. The average test AUC for the replicate runs is 0.966, and the standard deviation is 0.008.

Average Sensitivity vs. 1 – Specificity for *Gopherus berlandieri*



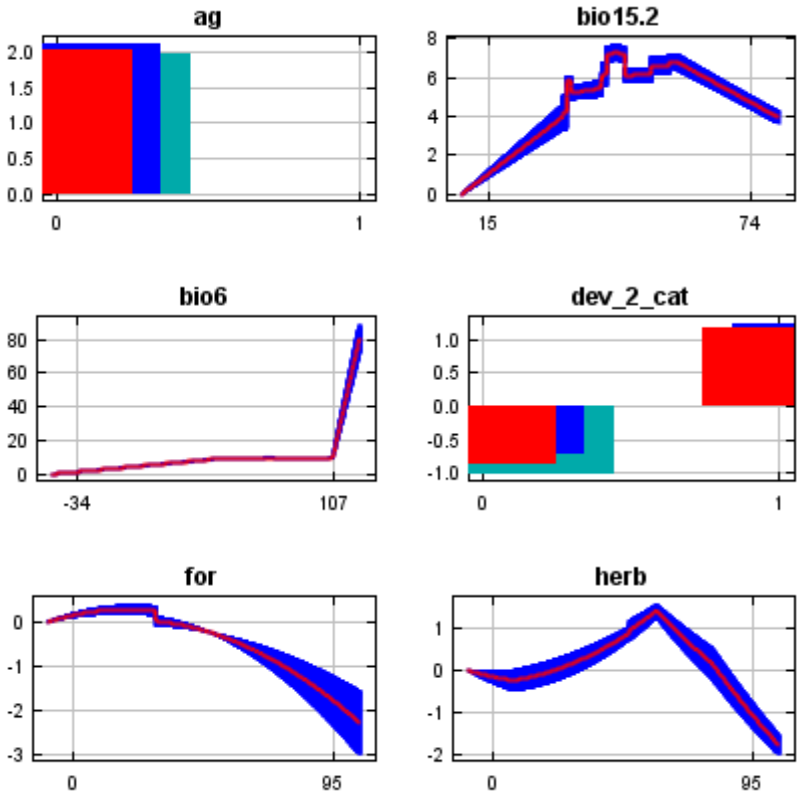
The following two pictures show the point-wise mean and standard deviation of outputs.

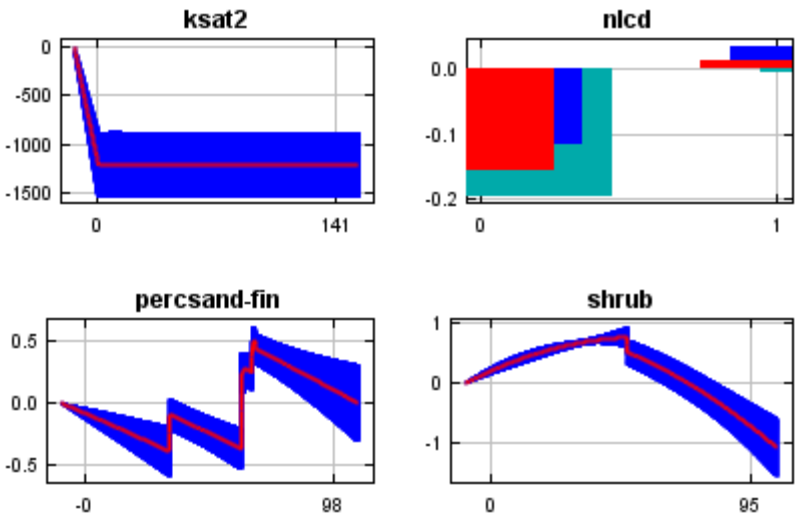




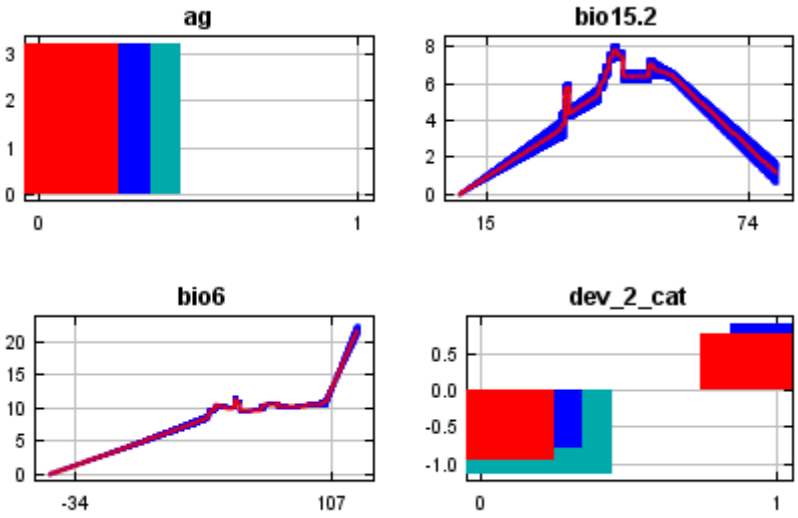
Response curves

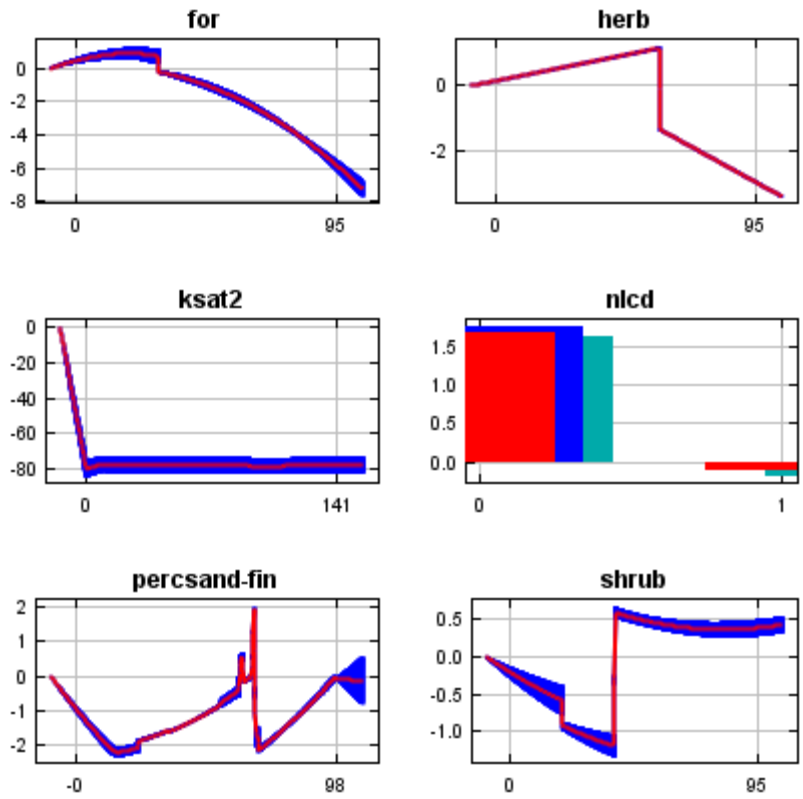
These curves show how each environmental variable affects the Maxent prediction. The (raw) Maxent model has the form exp(...)/constant, and the curves show how the exponent changes as each environmental variable is varied, keeping all other environmental variables at their average sample value. Note that the curves can be hard to interpret if you have strongly correlated variables, as the model may depend on the correlations in ways that are not evident in the curves. In other words, the curves show the marginal effect of changing exactly one variable, whereas the model may take advantage of sets of variables changing together. The curves show the mean response of the 3 replicate Maxent runs (red) and and the mean +/- one standard deviation (blue, two shades for categorical variables).





In contrast to the above marginal response curves, each of the following curves represents a different model, namely, a Maxent model created using only the corresponding variable. These plots reflect the dependence of predicted suitability both on the selected variable and on dependencies induced by correlations between the selected variable and other variables. They may be easier to interpret if there are strong correlations between variables.



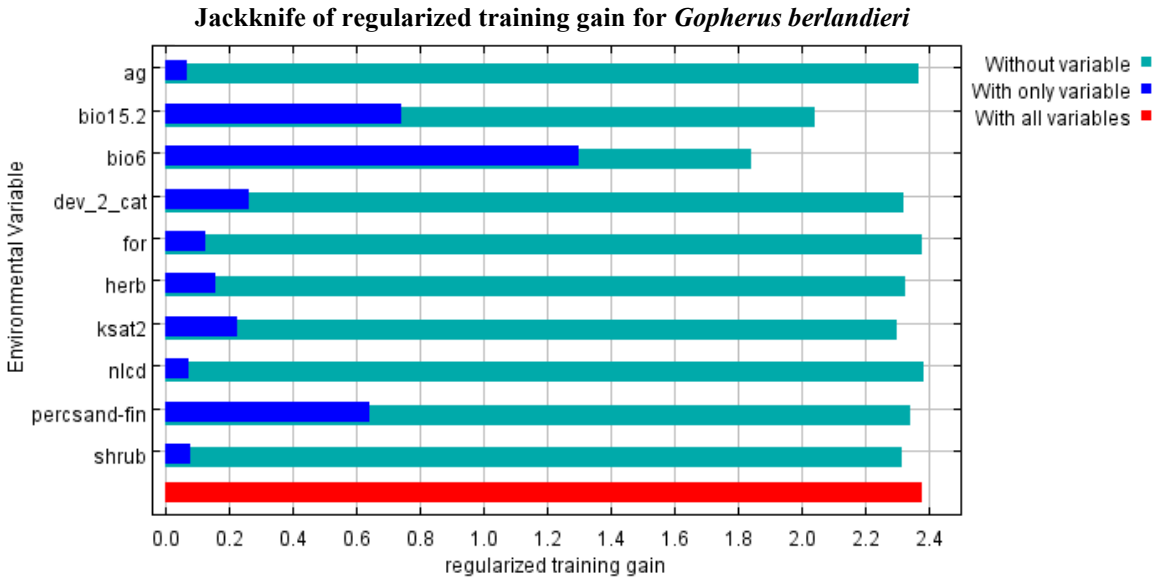


Analysis of variable contributions

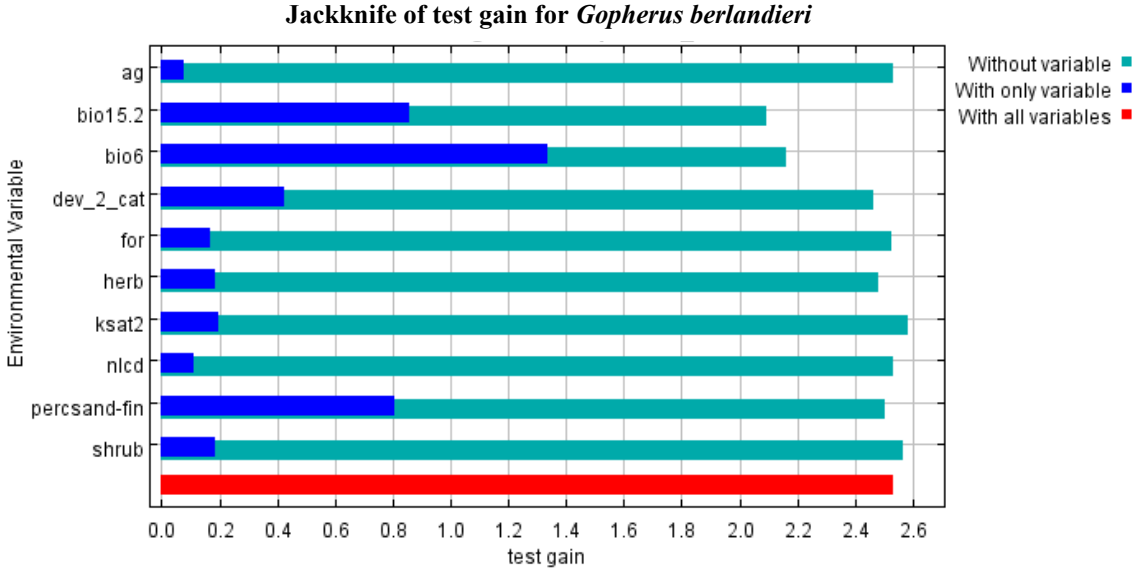
The following table gives estimates of relative contributions of the environmental variables to the Maxent model. To determine the first estimate, in each iteration of the training algorithm, the increase in regularized gain is added to the contribution of the corresponding variable, or subtracted from it if the change to the absolute value of lambda is negative. For the second estimate, for each environmental variable in turn, the values of that variable on training presence and background data are randomly permuted. The model is reevaluated on the permuted data, and the resulting drop in training AUC is shown in the table, normalized to percentages. As with the variable jackknife, variable contributions should be interpreted with caution when the predictor variables are correlated. Values shown are averages over replicate runs.

Variable	Percent contribution	Permutation importance
bio6	47.6	56.7
bio15.2	25.8	24.9
dev_2 cat	10.2	6.1
ksat	6.8	5.7
percsand	3.4	0.9
herb	2.8	1.2
ag	2.2	1.8
shrub	1.1	2.4
for	0.2	0.3
nlcd	0	0.1

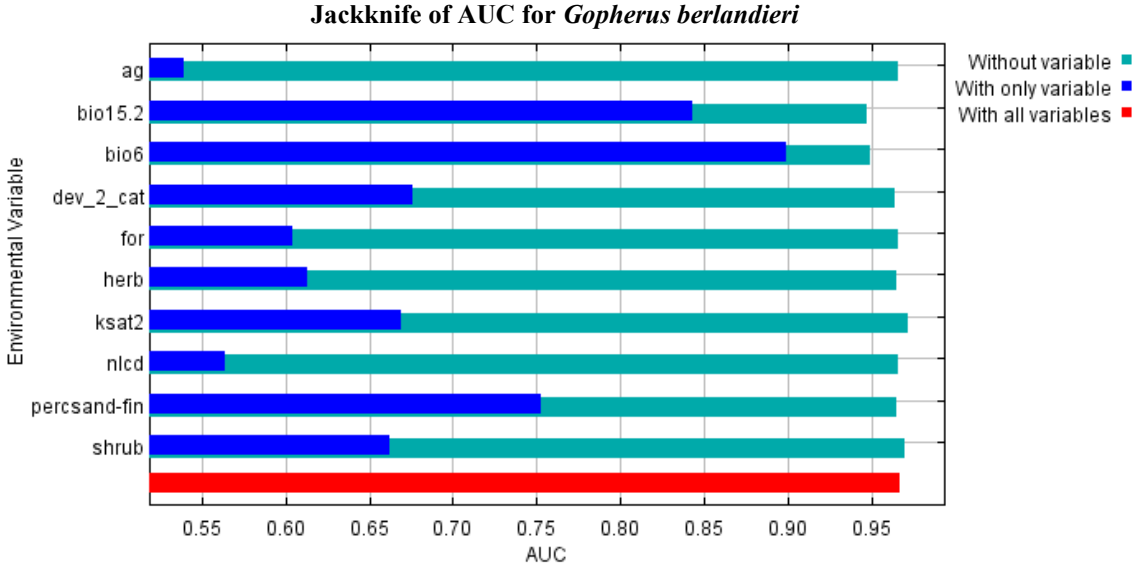
The following picture shows the results of the jackknife test of variable importance. The environmental variable with highest gain when used in isolation is bio6, which therefore appears to have the most useful information by itself. The environmental variable that decreases the gain the most when it is omitted is bio6, which therefore appears to have the most information that isn't present in the other variables. Values shown are averages over replicate runs.



The next graph shows the same jackknife test, using test gain instead of training gain. Note that conclusions about which variables are most important can change, now that we're looking at test data.



Lastly, we have the same jackknife test, using AUC on test data.



Command line to repeat this species model: java density.MaxEnt nowarnings noprefixes -E "" -E Gopherus_berlandieri responsecurves jackknife outputdirectory=F:\Anjana\output-may-2015\may7-subsample-3reps-10m samplesfile=D:\Anjana\samples\180pts_10mts.csv environmentallayers=D:\Anjana\new-maxent.cache randomseed nowarnings noaskoverwrite noremoveduplicates nowriteclampgrid nowritemess randomtestpoints=20 biasfile=D:\Anjana\Gopherus_berlandieri_bias_output\gb_bias_repr.asc replicates=3 replicatetype=subsample writebackgroundpredictions responsecurvesexponent writeplotdata nodoclamp appendtoresultsfile biastype=3 nocache allowpartialdata -N dev_cont -t ag -t dev_2_cat -t nlcd

LITERATURE CITED

Andersen, M. D., and G. P. Beauvais. 2013. Predictive Distribution Modeling of Species of Greatest Conservation Need in Texas. Report prepared by the Wyoming Natural Diversity Database, Laramie Wyoming, for the Texas Natural Diversity Database, Texas Parks and Wildlife Department, Austin, TX. 31.

Anderson, R. P, M. Dudík, S. Ferrier, A. Guisan, R. J. Hijmans, F. Huettmann, and N.E. Zimmermann. 2006. Novel methods improve prediction of species' distributions from occurrence data. Ecography 29(2): 129-151.

Auffenberg, W., and W. G. Weaver Jr. 1969. *Gopherus berlandieri* in southeastern Texas. Bulletin Florida State Museum 13:141-203.

Agassiz, L. 1857. Contribution to the natural history of the United States of America. Boston Little, Brown and Co. 196.

Baldwin, R. A. 2009. Use of maximum entropy modeling in wildlife research. Entropy 11(4): 854-866.

Berry, K. H., and M. J. Aresco. 2014. Threats and Conservation Needs for North American Tortoises. In Rostal, D.C, McCoy, E. D, and Mushinsky, H. R. (Ed.), Biology and Conservation of North American Tortoises: 127-133. John Hopkins University Press, Maryland.

Brown, J. L. 2014. SDMtoolbox: a python-based GIS toolkit for landscape genetic, biogeographic and species distribution model analyses. Methods in Ecology and Evolution 5(7): 694-700.

Bury, B., and E.L. Smith. 1986. Aspects of the ecology and management of the Tortoise *Gopherus berlandieri* at Laguna Atascosa, Texas. The Southwestern Naturalist 31: 387- 394.

Cagle, F. R. 1939. A system of marking turtles for future identification. Copeia 1939(3): 170-173.

Clostio, R. W., A. M., Martinez, K.E. LeBlanc, and N. M. Anthony. 2012. Population genetic structure of a threatened tortoise across the south-eastern United States: implications for conservation management. Animal Conservation 15(6): 613-625.

Diemer, J. E. 1986. The ecology and management of the gopher tortoise in the southeastern United States. Herpetologica 125-133.

Dixon, J.R. 2013. Amphibians and reptiles of Texas: With keys, taxonomic synopses, bibliography, and distribution maps (W. L. Moody Jr. Natural History Series). Texas A&M University Press. Third Edition.

Earl, D. A. and B. M. vonHoldt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4(2): 359-361

Edwards, T., C.J. Jarchow, C.A. Jones, and K. E. Bonine. 2010. Tracing genetic lineages of captive desert tortoises in Arizona. The Journal of Wildlife Management 74(4): 801-807.

Edwards, T, and J. S. Harrison. 2014. Population and Conservation Genetics of North American Tortoises. In Rostal, D.C, E. D. McCoy, and H. R. Mushinsky, (Ed.), Biology and Conservation of North American Tortoises: 127-133. John Hopkins University Press, Maryland.

Esque, T. C., C. R. Schwalbe, L. A. Defalco, R. B. Duncan, and T. J. Hughes. 2003. Effects of desert wildfires on desert tortoise (*Gopherus agassizii*) and other small vertebrates. The Southwestern Naturalist 48(1): 103-111.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular ecology 14(8): 2611-2620.

Excoffier, L., G. Laval and S. Schneider. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47.

Fielding, A. H., and J. F. Bell. 1997. A review of methods for the assessment of prediction errors in conservation presence/absence models. Environmental Conservation 24(01): 38-49.

Fujii, A., and M. R. Forstner. 2010. Genetic variation and population structure of the Texas tortoise, *Gopherus berlandieri* (Testudinidae), with implications for conservation. Chelonian Conservation and Biology, 9(1): 61-69.

Gillen, J. L., and E. Kiviat. 2012. Environmental reviews and case studies: Hydraulic fracturing threats to species with restricted geographic ranges in the Eastern United States. Environmental Practice 14: 320-331.

Grendár, M., and M. Grendár. 2001. Maximum entropy: Clearing up mysteries. Entropy 3(2): 58-63.

Groombridge, M. 1982. The IUCN Amphibia-Reptilia Red Data Book Part 1: Testudines, Crocodylia, Rynchocephalia. Gland, Switzerland IUCN. 426.

Hamilton, R. D. 1944. Notes on mating and migration in Berlandier's turtle. Copeia. 1944: 62.

Hijmans, R.J., S. E. Cameron, J. L. Parra, P.G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25: 1965-1978.

Hirzel, A. H., V. Helfer, and F. Metral, 2001. Assessing habitat-suitability models with a virtual species. Ecological Modelling 145: 111-121.

Jahrsdoerfer, S. E., and D. M. Leslie Jr. 1988. Tamaulipan brushland of the Lower Rio Grande Valley of South Texas: Description, human impacts, and management options. Oklahoma Cooperative Fish and Wildlife Research Unit, Stillwater.

Judd, F. W. and F. L. Rose. 1983. Population structure, density and movements of the Texas Tortoise *Gopherus berlandieri*. The Southwestern Naturalist 28: 387-398.

Judd, F. W. and F. L. Rose. 1989. Egg production by the Texas Tortoise, *Gopherus berlandieri*, in southern Texas. Copeia 588-596.

Kreiser, B. R., J. R. Ennen, D. L. Gaillard, and C. P. Qualls. 2013. Isolation and characterization of polymorphic microsatellite loci for the gopher tortoise (*Gopherus polyphemus*) and cross-amplification in other species of *Gopherus*. Conservation Genetics Resources 5(1): 255-259.

Lamb, T., and C. Lydeard. 1994. A molecular phylogeny of the gopher tortoises, with comments on familial relationships within the Testudinoidea. Molecular Phylogenetics and Evolution 3(4): 283-291.

Murphy, R. W., K. H. Berry, T. Edwards, and A. M. McLuckie. 2007. A genetic assessment of the recovery units for the Mojave population of the desert tortoise, *Gopherus agassizii*. Chelonian Conservation and Biology 6(2).

Osentoski, M. F., and T. Lamb. 1995. Intraspecific phylogeography of the gopher tortoise, *Gopherus polyphemus*: RFLP analysis of amplified mtDNA segments. Molecular Ecology 4(6): 709-718.

Palma, L., P. Beja, and M. Rodrigues. 1999. The use of sighting data to analyze Iberian lynx habitat and distribution. Journal of Applied Ecology 36: 812–824.

Phillips, S. J., M. Dudík, and R. E. Schapire. 2004. A maximum entropy approach to species distribution modeling. In Proceedings of the twenty-first international conference on Machine learning (p. 83). ACM.

Phillips, S. J., R. P. Anderson, and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190(3): 231-259.

Phillips, S. J., and M. Dudík. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography 31(2): 161-175.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155(2): 945-959.

Pritchard, J. K., and W. Wen. 2010. Documentation for STRUCTURE software: Version 2.3. Available from http://pritch.bsd.uchicago.edu.

Ramirez, J.R. 1986. Water development projects in the Rio Grande and their relationships to the Santa Ana and Rio Grande Valley National Wildlife Refuges. A report prepared for Santa Ana and Rio Grande Valley National Wildlife Refuges, Alamo, Texas; by U.S. Fish and Wildlife Service, Ecological Services, Corpus Christi Field Office, Corpus Christi, Texas.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 223-225.

Rose, F. L. and F. W. Judd. 2014. The Texas Tortoise: A Natural History. University of Oklahoma Press: Norman.

Rose, F. L. and F. W. Judd. 1982. Biology and status of Berlandier's tortoise (*Gopherus berlandieri*). In: Bury, R. B. (Ed.). North American Tortoises: Conservation and Ecology U.S.D.I. Fish and Wildlife Service Wildlife Research Report 12.

Rose, F. L. and F. W. Judd. 1975. Activity and home range size of the Texas Tortoise, *Gopherus berlandieri*, in south Texas. Herpetologica 31:448-456

Sanchez-Zapata, J. A., and J. F. Calvo. 1999. Raptor distribution in relation to landscape composition in semi-arid Mediterranean habitats. Journal of Applied Ecology 36: 254–262.

Schwartz, T. S., and S. A. Karl. 2005. Population and conservation genetics of the gopher tortoise (*Gopherus polyphemu*s). Conservation Genetics 6(6): 917-928.

Schwartz, T. S., M. Osentoski, T. Lamb, and S. A. Karl. 2003. Microsatellite loci for the North American tortoises (genus *Gopherus*) and their applicability to other turtle species. Molecular Ecology Notes 3(2): 283-286.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18(2): 233-234.

Store, R., and J. Jokimäki. 2003. A GIS-based multi-scale approach to habitat suitability modeling. Ecological Modelling 169(1): 1-15.

Warren, D. L., R. E. Glor, and M. Turelli. 2010. ENMTools: a toolbox for comparative studies of environmental niche models. Ecography 33(3): 607-611.

Warren, D. L., and S. N. Seifert. 2011. Ecological niche modeling in Maxent: the importance of model complexity and the performance of model selection criteria. Ecological Applications 21(2): 335-342.

Young, N., L. Carter, and P. Evangelista. 2011. A MaxEnt Model v3.3.3 e Tutorial (ArcGIS v10). Natural Resource Ecology Laboratory at Colorado State University and the National Institute of Invasive Species Science, Colorado, USA.