DEMOGRAPHY AND POPULATION STRUCTURE OF A RIO GRANDE ENDEMIC EMYDID THE BIG BEND SLIDER

DISSERTATION

Presented to the Graduate Council of
Texas State University-San Marcos
in Partial Fulfillment
of the Requirements

for the Degree
Doctor of PHILOSOPHY

by

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San Marcos, Texas
August 2010

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ACKNOWLEDGEMENTS

I would like to thank my parents for their support and encouragement. I would also like to thank the many other students that I had the privilege to collaborate with and learn from over the years. Most importantly, I would like to thank my dear and very patient wife, Keri, for her unending support. I couldn't have done it without you.

This manuscript was submitted on 19 August, 2010.

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ABSTRACT

DEMOGRAPHY AND POPULATION STRUCTURE OF

A RIO GRANDE ENDEMIC EMYDID

THE BIG BEND SLIDER

by

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December 2010

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The once mighty Rio Grande has become one of the most challenged river systems in North America, and has been included in a list of the 10 rivers most at risk globally. Knowledge of the status and structure of current populations is critical for management and conservation of native species. The Big Bend slider, *Trachemys gaigeae gaigeae*, is a Rio Grande endemic on the IUCN red list and has apparently been extirpated from most of its historic range in the Rio Grande. This species is also facing competition and hybridization with introduced

individuals of its sister taxon, *Trachemys scripta elegans* (Red-eared slider). Both mitochondrial DNA and 13 nuclear microsatellite markers were employed to examine these issues. Analysis of mitochondrial data from *Trachemys* have shown that hybridization is indeed occurring in extant *T. g. gaigeae* populations. Microsatellite data also support occurrence of hybridization, viability of hybrids, and supports introduced origin of *T. s. elegans* in Big Bend National Park but also revealed potential range expansion of south Rio Grande native *Trachemys scripta elegans*. Microsatellite analysis results for *Trachemys* show evidence of historic gene flow between New Mexico and Texas populations, though they are now well differentiated. Genetic diversity is lower in *T. g. gaigeae* than in *T. s. elegans*. Mark-recapture data collected over a five-year span for *T. gaigeae* have demonstrated that the species has a greater than anticipated ability for dispersal yet shows a high degree of site fidelity even after large floods. Abundance estimates from mark-recapture data show that the Big Bend population is small, and this is corroborated by genetic estimates of effective population size. Results provided by this study allow *T. g. gaigeae* populations to be monitored in the future and demonstrate that *T. g. gaigeae* is a species of high conservation concern.

CHAPTER I

INTRODUCTION

One of the greatest global challenges facing conservation biologists today is reducing the accelerated rate of species loss witnessed in the last century. There are multiple causes for the loss of species. Many are anthropogenic: habitat destruction and fragmentation, pollution, and commercial exploitation all exact a heavy toll. A more recently recognized anthropogenic cause of species loss is the introduction of exotic species into new areas, which can have drastic effects on native flora and fauna (Pimentel et al., 2005; Quian and Ricklefs, 2006). Remote areas are not spared and all of these factors are impacting the flora and fauna of the Chihuahuan desert (Hoyt, 2002). Big Bend National Park, situated along the Rio Grande on the Texas/Mexico border, is the largest intact contiguous portion of the Chihuahuan desert in the United States. While the desert environment appears harsh, it houses extraordinary biodiversity (Hoyt, 2002).

Big Bend National Park encompasses a vast area of the Chihuahuan desert bordered by the Rio Grande to the south. This river is often the only water available for many miles and the river ecosystem itself was listed as one of the 10 rivers most at risk worldwide due to overdraw of water (Wong et al., 2007). Given the climbing anthropogenic pressures on this eco-region, the fate of many

Rio Grande endemic species may lie in populations inside regions, such as Big Bend National Park, that receive state and/or federal protection. One such species is the Big Bend slider (*Trachemys gaigeae gaigeae*), an Emydid turtle that was once common throughout the Rio Grande River from west Texas into New Mexico, and the Rio Conchos in Mexico (Ernst et al., 1994). This turtle is a unique lineage of slider (Jackson et al., 2008) with a small geographic distribution in compromised habitat, and after evaluation by the World Conservation Union (IUCN) it was placed on the Red List as a species vulnerable to extinction due to range contraction and loss of habitat (Baillie and Groombridge, 1996). Because of the impacts to the watershed, the distribution of this species has been undergoing fragmentation for some time (Ernst et al., 1994). Currently, there are several large gaps in its distribution along this section of the river. Surveys in the late 1990's documented that *T. g. gaigeae* has been extirpated from the larger portion of its historic distribution (Forstner et al., 1999). The largest extant population is largely contained within the boundaries of Big Bend National Park, which also represents the largest contiguous area of *T. g. gaigeae* habitat that is under protection. Smaller populations are scattered from Elephant Butte reservoir to the Bosque del Apache National Wildlife Refuge in New Mexico, and recently a small remnant population was detected in Hudspeth County, Texas, which lies between the Big Bend and Elephant Butte populations (Forstner et al., in press). Virtually no data exist for the evaluation of the species in the Rio Conchos in Mexico (the major tributary of the Rio Grande upstream of Big Bend

National Park near Presidio). This tributary is assumed to have held a similarly abundant population, but is also likely to have suffered the same consequences resulting from anthropogenic pressures such as dam construction, flow reduction, and channelization. The long-term consequences of these changes to the river on the watershed as an ecosystem are not clear nor have the impacts to Rio Grande endemics like the Big Bend slider been quantified. If enough of the habitat is lost or made unsuitable, historically contiguous populations can become fragmented and isolated, resulting in reduced or interrupted gene flow with attendant losses to genetic diversity (Gerlach and Musolf, 2000).

The genus *Trachemys* is a species-rich group of turtles in the family Emydidae. Species of this genus are spread throughout North, Central and South America as well as the Caribbean Islands. Most members of this genus have historically been placed into the ambiguously defined *T. scripta* complex, attributed to the fact that few members of this genus occur in sympatry (Seidel, 2002). More recent studies, especially those involving molecular techniques, have suggested that some, if not many, of these subspecies should be elevated to species level (Stephens and Wiens, 2003). The interspecific relationships within this genus have remained largely unresolved (Seidel and Smith, 1986), and it has been suggested that a more comprehensive phylogenetic analysis of the genus was needed to resolve these issues (Seidel, 2002; Seidel et al., 1999), especially in the case of the *T. scripta* complex (Seidel et al., 1999; Stephens and Wiens, 2003). Part of the present study using mitochondrial DNA has

established the monophyly of a North American lineage including *T. g. gaigeae* and its sister taxon, *T. scripta elegans* (red-eared slider) (Jackson et al., 2008). The red-eared slider is a common pond turtle that is often sold in the pet trade. The introduction of red-eared sliders has adversely affected populations of other turtle species around the globe as they are strong competitors and have shown an ability to reduce survivorship of other turtle species (Cadi and Joly, 2004; Chen and Lue, 1998; Luiselli et al., 1997). The colonizing and competitive abilities of the red-eared slider have led to its inclusion in the IUCN list of the world's 100 most problematic invasive species (Lowe et al., 2000). Red-eared sliders are remarkably successful invaders worldwide, having been found in ten U.S. states and 21 countries outside their native range (Moll and Moll, 2004). They are not considered native to the Big Bend area but have been introduced, most likely by people releasing their pet turtles when they no longer choose to care for them (Dixon, 2002; Spinks et al., 2003). The introduction of the red-eared slider *T. s. elegans* poses a unique threat to *T. g. gaigeae* populations both in the Rio Grande of New Mexico and in the Rio Grande of Texas. It has been suggested that hybridization between *T. g. gaigeae* and *T. s. scripta* in New Mexico only occurs where *T. s. elegans* is introduced (Degenhardt et al., 1996), which may also be the case in Big Bend National Park (Forstner et al., in press). Hybridization may lead to loss of genetic diversity in *T. g. gaigeae*, and possibly to the replacement of parts of their genome by portions of the *T. s. elegans* genome (i.e., introgression). This is a danger particularly to turtles, which have

already been shown to possess lower levels of genetic diversity than other organisms (Avise et al., 1992). In addition, these processes can contribute to the loss of a species, especially a rare taxon (Rhymer and Simberloff, 1996). Molecular markers can be used to evaluate the extent of such hybridization (Scribner et al., 2001) and the extent of any introgression (Gerber et al., 2001) allowing appropriate management strategies to be implemented to preserve the genetic integrity of *T. g. gaigeae*. Recent advances in statistical algorithms have allowed hybrids to be detected using microsatellites; however, determining the extent of hybridization, especially the detection of backcrossed individuals who are less likely to be phenotypically distinct, is difficult and can be largely dependent upon the number of markers used (Juha-Pekka and Primmer, 2006). It has also been demonstrated that the factor having the greatest effect on the ability of these algorithms to accurately detect hybrids is the frequency of hybrids in the population and in the sample.

This study addresses a number of hypotheses relating to the Rio Bravo watershed of the Chihuahuan desert. Molecular data from *T. g. gaigeae* were used to evaluate the genetic differentiation between populations in Texas and New Mexico. Molecular methods were also used to determine if *T. s. elegans* within the extant range of *T. g. gaigeae* in Texas or New Mexico are introduced (as hypothesized). An attempt was made to identify the region(s) of origin of the introduced populations of *T. s. elegans*, as well as further elucidate the

relationship between this species and *T. g. gaigeae*. The extent of hybridization of these two species was also addressed.

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CHAPTER II

A MITOCHONDRIAL DNA PHYLOGENY OF EXTANT SPECIES OF THE GENUS *TRACHEMYS* WITH RESULTING TAXONOMIC IMPLICATIONS

The genus *Trachemys* is a speciose group of turtles in the family Emydidae. Species of this genus are spread throughout North, Central and South America as well as the Caribbean Islands. Most members of this genus were historically placed into the ambiguously defined *T. scripta* complex, which has been attributed to the fact that few members of this genus are sympatric (Seidel 2002). More recent studies have argued that some, if not many, of these are likely to actually be species rather than subspecies (Stephens and Wiens 2003). If the species designation of many of the subspecies is correct, then the interspecific relationships within this genus are largely unresolved, and a more comprehensive phylogenetic analysis of the genus is needed to resolve these issues (Seidel et al. 1999, Seidel 2002). This is especially true in the case of the *T. scripta* complex (Seidel et al. 1999, Stephens and Wiens 2003). We use the taxonomy proposed by Seidel et al. (2002) to avoid confusion among historical species/subspecies. In this study, DNA sequence data from the NADH 4 region and flanking tRNAs of 52 individuals of 18 species and subspecies in *Trachemys* were analyzed by maximum parsimony, maximum likelihood and Bayesian

analysis methods. Our explict goal is to provide an mtDNA phylogeny which includes sequence data for a majority of the currently described taxa. Particular emphasis was given to the North American species group, specifically the relationship and validity of *Trachemys gaigeae*.

MATERIALS AND METHODS

Blood samples were collected from wild caught, pet trade, and zoo animals by various individuals (mainly MRJF, DES, and JRD). Some specimens were accessioned into museum collections, and some remain in private collections (Table 1). Remaining blood and/or DNA samples are in the MRJ Forstner frozen tissue collection at Texas State University-San Marcos.

Blood was isolated from each individual and stored in Blood Storage Buffer (100 mM Tris pH 8.0, 100 mM Na_2EDTA, 10 mM NaCl, and 1% SDS) until needed. DNA was extracted from fresh/frozen tissue or whole blood using the proteinase K protocol of (Maniatis et al. 1982), as modified by (Hillis and Davis 1986). The primers used in PCR amplification were obtained from Arévalo et al., (1994). The primers ND4 and Leucine were chosen because they show a high degree of conservation within turtle sequences and were shown to be phylogenetically informative in squamates (Arevalo et al. 1994, Forstner et al. 1995). A 992 basepair (bp) fragment of mtDNA is amplified by these primers and contains the last 768 bases of the ND4 gene and the tRNAs: Histidine, Serine, and Leucine. Sequencing reactions were performed using the Applied Bio-

Systems, Incorporated (ABI) Dideoxy termination cycle sequencing kit in conjunction with an ABI 373A automated sequencer.

All sequences were aligned using MacClade 4 (Madison and Madison 2003). All sequences from individuals of the same species that were identical were collapsed into a single sequence, again using MacClade. This resulted in a data set of 54 individual sequences from 20 taxa. All sequences used in this analysis were accessioned into NCBI GenBank (DQ338474-DQ338527). A partition homogeneity test was conducted using PAUP* 4b10 (Swofford 2002) to determine if it would be necessary to partition the tRNA's and the protein coding fragment of ND4. Modeltest 3.5 (Posada and Crandall 1998) was used to determine the appropriate model of sequence evolution for this data set under the AIC criteria (Posada and Buckley 2004) with four different outgroup arrangements. The outgroups tested were *Testudo kleinmanni* only; *Testudo* and *Pseudemys texana; Testudo*, *Heosemys*, *Sacalia*, and *Callagur;* and finally *Pseudemys*, *Testudo*, *Heosemys*, *Sacalia*, and *Callugur*. Neighbor joining analyses were conducted using Maximum Likelihood Estimate (MLE) distance settings corresponding to the results of the model selection process for each outgroup arrangement, and the results were compared in order to ascertain sensitivity of the data to outgroup selection. All four outgroup arrangements resulted in the selection of the same model in Modeltest 3.5 (GTR + G) and produced analogous neighbor joining topologies using MLE distances. Thus, the data set was not sensitive to outgroup selection and a single outgroup

arrangement was chosen (*Testudo kleinmanni* and *Pseudemys texana*), providing a distantly related taxon, as well as a proximal sister taxon within the same family.

The model selected by Modeltest (GTR + G) was then used in maximum likelihood analysis of the dataset in PAUP*. The parameter estimates from Modeltest were used in this analysis. The resulting ML topology was bootstrapped (1000 replicates) to evaluate support of the relationships proposed.

MrModeltest was used to determine the most appropriate model using AIC (GTR+G) for Bayesian analysis using MrBayes (Huelsenbeck and Ronquist 2001). An MCMC analysis was conducted in MrBayes using the GTR+G model to implement a "best" model. This analysis was run for $1x10^6$ generations, sampling every 100, with one cold and three hot chains. A burn in of 300 samples (sumt burnin=300) was determined to be appropriate from stabilization of a log likelihood plot, and posterior probabilities for the resulting topology were calculated using PAUP*.

A partitioned Bayesian analysis was also conducted using MrBayes. The data set was divided into four partitions, one for each codon position in the protein coding ND4 portion, and the fourth partition contained the tRNAs. Each partition was independently run through MrModeltest, and the best model for each partition selected by AIC. The selected model and parameter estimates for each partition were then input in MrBayes. 6 chains (5 hot, 1 cold) were run for $3x10^6$ generations, sampling every 1000 generations. The first 25 % of the

samples were discarded, equivalent to a burn in of 750 samples. Posterior probabilities for the resulting topology were calculated using PAUP*.

Parsimony analyses were conducted using PAUP*. The most parsimonious tree for the dataset was found using a full heuristic search with simple stepwise addition and tree bisection-reconnection (TBR). The result was then subjected to a non-parametric bootstrap as implemented in PAUP*, for 1000 replications with 10 TBR steps each, and the resulting 50% consensus topology was retained.

RESULTS

The result of the partition homogeneity test was not significant (p=0.15), therefore partitioning of the data set was not required. Modeltest selected GTR+G as the most appropriate single model for the dataset. Base frequencies for A, C, G, and T were 0.3513, 0.2635, 0.1305, and 0.2547 respectively. The rate variation followed a gamma distribution with a shape parameter of 0.4655, and there were 4 rate categories, and 6 substitution types. For the partitioned dataset, MrModeltest selected the GTR model for the first codon position, HKY+I for the second position, and GTR+G for the third position partition. HKY+G was selected for the tRNA partition. The results of the ML (Figure 1) and Bayesian (Figure 2) analyses were generally congruent. The results are generally congruent with the taxonomy of Seidel (2002). Both topologies support the significance of *T. gaigeae*, *T. emolli*, *T. taylori*, *T. yaquia*, *T. dorbigni*, *T. terrapen* and *T. decussata* lineages. The results of both analyses also show clearly

resolved North American (*T. scripta scripta, T. s. troostii, T. s. elegans,* and *T. gaigeae*), Meso-American (*T. emolli, T. taylori, T. venusta venusta, T. v. cataspila, T. v. grayi, T. callirostris callirostris, T. c. chichiriviche, T. yaquia,* and *T. dorbigni*) and West Indian (*T. decorata, T. stejnegeri stejnegeri, T. stegnegeri vicina, T. terrapen, T. decussata decussata* and *T. d. angusta*) monophyletic units.

DISCUSSION

While the three main monophyletic (North, Meso-American, and West Indian) lineages apparent in the results of these analyses are generally consistent with the results of other studies (Seidel 2002, Stephens and Wiens 2003), there is incongruence regarding the relationships among some species. The analyses of Stephens and Wiens (2003) placed *T. gaigeae* in a clade with species from South America and Mexico, while our analyses places them as more closely related to the North American *T. scripta* complex, and as part of the monophyletic North American lineage. Our placement of *T. gaigeae* is strongly supported by both the MP and ML bootstrap values and Bayesian posterior probabilities from both partitioned and non-partitioned analyses (Figures 1 and 2). Together with the concept of the evolutionary significant unit (ESU) (Ryder 1986, Moritz 1994), which in some cases is the equivalent of a "species" (Moritz 1994), our analyses support the species status of *T. gaigeae* as proposed by several authors (Weaver and Rose 1967, Ward 1984, Seidel et al. 1999, Seidel 2002). Our intention here, however, is to recognize this lineage as unique and

worthy of treatment as a unit for conservation, rather than contribute to the overabundance of literature arguing the appropriate criteria for "species" definition. Our study failed to resolve the *Trachemys venusta* and *Trachemys callirostris* species complexes of Seidel (2002). However, the lack of phylogenetic resolution does not provide an inherent default hypothesis, and therefore Seidel's taxonomy is provisionally retained. These ambiguous relationships may eventually be resolved as more data are collected and analyzed.

In conclusion, it appears that when mtDNA data are considered, the taxonomy of *Trachemys* proposed by Seidel (2002) is the most reasonable for the genus. The proposed species status of *T. gaigeae* (Weaver and Rose 1967, Ward 1984, Seidel et al. 1999, Seidel 2002) is also supported by our data. In our evaluation of the specific status for this taxon we have sought to use historical evaluations in conjunction with supported results from our current mtDNA hypothesis. In our support for *T. gaigeae* we explicitly acknowledge our failure to more broadly evaluate the remaining potential evolutionarily significant units within this genus (Moritz 1994). This decision was made in keeping with the recent voucher manuscript (Lehn et al. 2007) in which we agree that significant systematic decisions should not be completed in the absence of traditional voucher specimens. We would still suggest, however, that in light of the concordance of most of the relationships in our analysis and those in the morphological analysis of Seidel (2002), his proposed taxonomy represents the best current working taxonomy of Trachemys. This makes the most use of what

data is available, and it appears from the analysis of this data that this taxonomic arrangement does the most to preserve the diversity contained within the genus by recognizing diagnosable lineages as unique.

ACKNOWLEDGEMENTS

We would like to thank Francis Rose, Todd Swannack, B. Schaffer and F. Pardham for review, comments, and suggestions used in the manuscript. We also thank S. K. Davis and T. Guerra for their long support of this project. We sincerely appreciate the efforts by those maintaining many of these taxa over many years in captivity, allowing us access to those collections, and their helpful discussions on the systematics of these animals. Finally, without the assistance and collaboration of the Texas Cooperative Wildlife Collection, TPWD, and NMGF we could not have completed the work. This work was supported in part by funding from the USGS and the NPS to MRJF.

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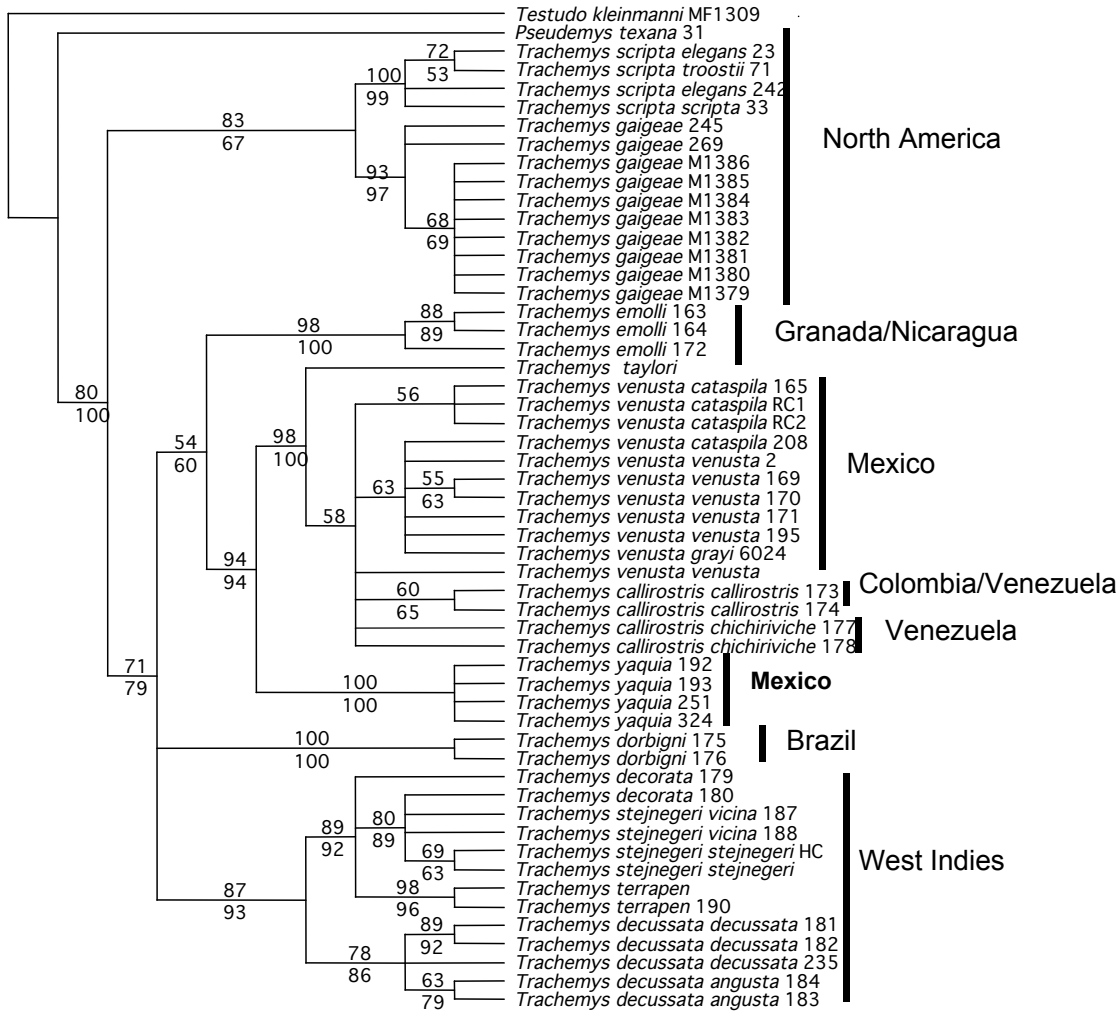

Figure 1. **Bootstrap consensus of maximum parsimony and maximum likelihood phylogenetic analyses.** Bootstrap consensus of the maximum parsimony and maximum likelihood analyses of ND4-leucine tRNA region of mitochondrial DNA in *Trachemys*. ML bootstrap support values are shown above supported branches, and MP bootstrap values are shown below. Major regional clades are illustrated to the right of taxon names.

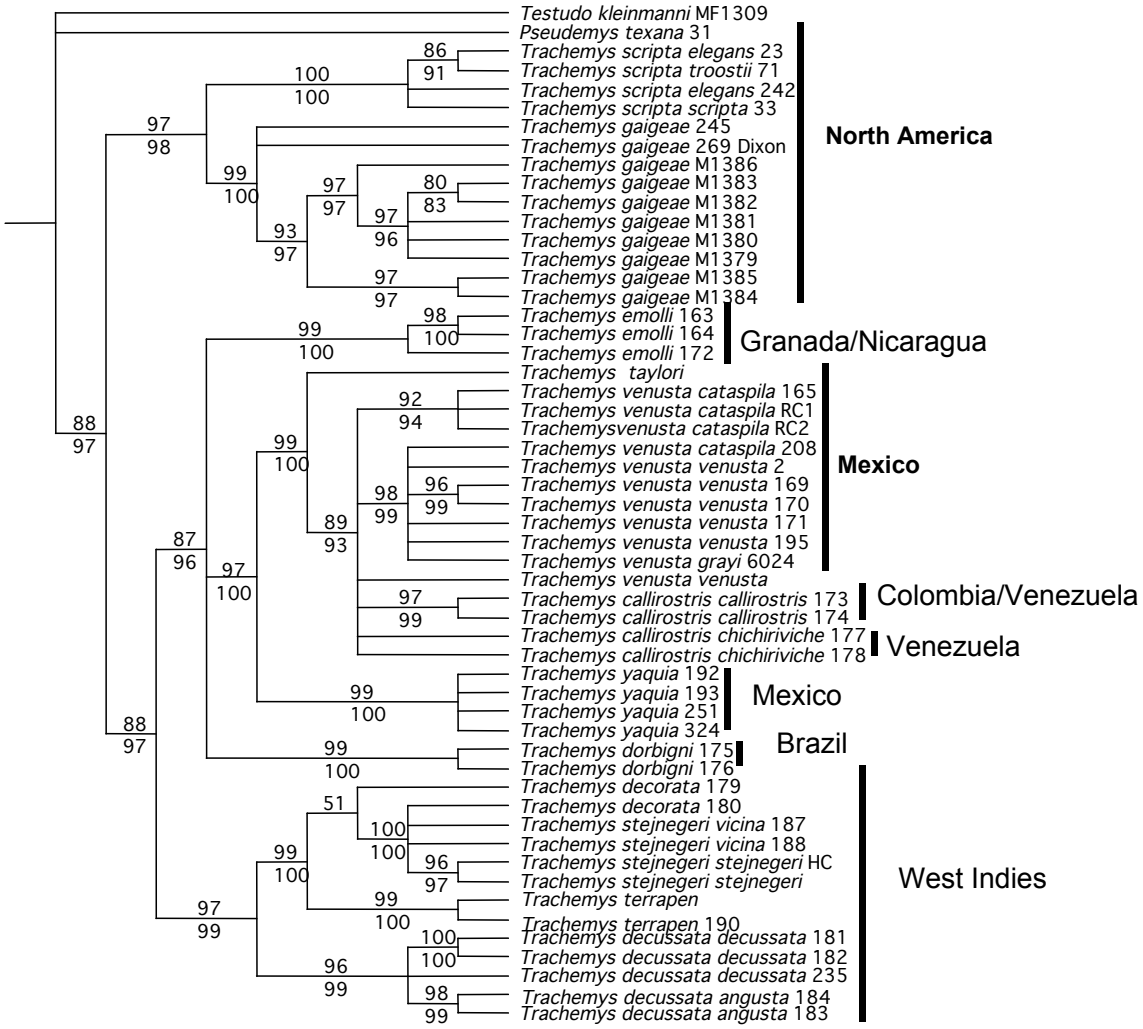

Figure 2. **Bayesian phylogenetic analyses.** Results of Bayesian analyses of the ND4-leucine tRNA region of mitochondrial DNA in *Trachemys*. Posterior probabilities from analysis using a single model are shown above supported branches, and the posterior probabilities from the partitioned analysis are shown below. Regional clades are illustrated to the right of taxon names.

Table 1. Specimen Data for all Taxa Used in Phylogenetic Reconstruction. Texas samples were collected under kind permission of the TPWD SPR-0290-022. Captive samples maintained under ESC8945 (PC1) and Tennessee collection made under license 2504378. Living voucher material will be deposited into the TCWC as available after normal mortality of the individuals.

NAME	GENBANK ACCESSION #	LOCATION	COLLECTION	MUSEUM #
Pseudemys texana 31	DQ338475	Colorado River, Travis County, Texas	TCWC	TCWC72324
Trachemys decorata 179	DQ338515	Pet Trade	Private collection 1	Living voucher
Trachemys decorata 180	DQ338516	Pet Trade		Blood only
Trachemys decussata angusta 183	DQ388521	Grand Cayman	released	Photo voucher
Trachemys decussata angusta 184	DQ388520	Grand Cayman	released	Photo voucher
Trachemys decussata decussata 181	DQ338517	Pet Trade	Private collection 1	Living voucher
Trachemys decussata decussata 182	DQ338518	Pet Trade	Private collection 1	Living voucher
Trachemys decussata decussata 235	DQ338519	Pet Trade		Blood only
Trachemys dorbigni 175	DQ338513	Uruguay	Private collection 1	Living voucher
Trachemys dorbigni 176	DQ338514	Uruguay	Private collection 1	Living voucher
Trachemys gaigeae 245	DQ338480	Dona Ana County, New Mexico	TCWC	TCWC72425
Trachemys gaigeae 269	DQ338481	Dona Ana County, New Mexico	TCWC	TCWC86270
Trachemys gaigeae M1379	DQ338489	Black Gap WMA, Brewster Co., TX	released	Photo voucher
Trachemys gaigeae M1380	DQ338488	Black Gap WMA, Brewster Co., TX	released	Photo voucher
Trachemys gaigeae M1381	DQ338487	Black Gap WMA, Brewster Co., TX	released	Photo voucher
Trachemys gaigeae M1382	DQ338486	Black Gap WMA, Brewster Co., TX	released	Photo voucher
Trachemys gaigeae M1383	DQ338485	Black Gap WMA, Brewster Co., TX	released	Photo voucher
Trachemys gaigeae M1384	DQ338484	Black Gap WMA, Brewster Co., TX	released	Photo voucher
Trachemys gaigeae M1385	DQ338483	Black Gap WMA, Brewster Co., TX	released	Photo voucher
Trachemys gaigeae M1386	DQ338482	Black Gap WMA, Brewster Co., TX	released	Photo voucher
Trachemys callirostris callirostris 173	DQ338504	Pet Trade	Private collection 1	Living voucher
Trachemys callirostris callirostris 174	DQ338505	Pet Trade	Private collection 1	Living voucher
Trachemys venusta cataspila 165	DQ338494	Northern Mexico	Private collection 1	Living voucher
Trachemys venusta cataspila 208	DQ338495	Northern Mexico	Private collection 1	Living voucher
Trachemys venusta cataspila RC1	DQ338496	unknown		Blood only

TABLE 1, CONTINUED

NAME	GENBANK ACCESSION #	LOCATION	COLLECTION	MUSEUM #
Trachemys venusta cataspila RC2	DQ338497	unknown		Blood only
Trachemys callirostris chichiriviche 177	DQ338506	Venezuela	Private collection 1	Living voucher
Trachemys callirostris chichiriviche 178	DQ338507	Venezuela	Private collection 1	Living voucher
Trachemys scripta elegans 23	DQ338476	Cameron County, Texas	TCWC	TCWC72426
Trachemys scripta elegans 242	DQ338477	Cameron County, Texas		Photo voucher
Trachemys emolli 163	DQ338490	Pet Trade	Private collection 1	Living voucher
Trachemys emolli 164	DQ338491	Pet Trade	Private collection 1	Living voucher
Trachemys emolli 172	DQ338492	Panama	Private collection 1	Living voucher
Trachemys scripta scripta 33	DQ338478	Dougherty County, Georgia	TCWC	TCWC72278
Trachemys scripta troostii 71	DQ338479	Bradley County, Tennessee		Blood only
Trachemys venusta venusta 169	DQ338500	Cozumel, Mexico	Private collection 1	Living voucher
Trachemys venusta venusta 170	DQ338501	Lake Bacalar, Belize	Private collection 1	Living voucher
Trachemys venusta venusta 171	DQ338502	Lake Bacalar, Belize	Private collection 1	Living voucher
Trachemys venusta venusta 195	DQ338503	New River, Belize	Private collection 1	Living voucher
Trachemys yaquia 192	DQ338509	Mexico	Private collection 1	Living voucher
Trachemys yaquia 193	DQ338510	Mexico	Private collection 1	Living voucher
Trachemys yaquia 251	DQ338511	Mexico	Private collection 2	Living voucher
Trachemys venusta grayi 6024	DQ338508	unknown		Blood only
Trachemys stejnegeri stejnegeri	DQ338527	Caguas, Puerto Rico	Private collection 1	Living voucher
Trachemys stejnegeri stejnegeri HC	DQ338526	Caguas, Puerto Rico	Private collection 2	Living voucher
Trachemys stejnegeri vicina 187	DQ338524	San Domingo, Dominican Republic	Private collection 1	Living voucher
Trachemys stejnegeri vicina 188	DQ338525	San Domingo, Dominican Republic	Private collection 1	Living voucher
Trachemys taylori	DQ338493	unknown	Private collection 2	Living voucher
Trachemys terrapen	DQ338522	Ocho Rios, Jamaica	Private collection 1	Living voucher
Trachemys terrapen 190	DQ338523	Ocho Rios, Jamaica	Private collection 1	Living voucher
Trachemys venusta venusta	DQ338498	New River, Belize		Blood only
Trachemys venusta venusta 2	DQ338499	New River, Belize		Blood only
Trachemys yaquia 324	DQ338512	Mexico	Private collection 2	Living voucher

CHAPTER III

HYBRIDIZATION BETWEEN THE BIG BEND SLIDER (*TRACHEMYS GAIGEAE GAIGEAE*) AND RED-EARED SLIDERS (*TRACHEMYS SCRIPTA ELEGANS*) IN THE RIO GRANDE

The Big Bend slider (*Trachemys gaigeae gaigeae*) is an emydid turtle that was once common throughout the Rio Grande River from west Texas into New Mexico. This turtle is a unique lineage of slider with a small geographic distribution, in compromised habitat and, after evaluation by the World Conservation Union (IUCN), was placed on the Red List as a species vulnerable to extinction due to range contraction and loss of habitat (Baillie and Groombridge, 1996). One of the potential threats *T. g. gaigeae* populations face in both the Rio Grande of New Mexico and in the Rio Grande of Texas is a consequence of the introduction of the red-eared slider, *Trachemys scripta elegans*. The red-eared slider is a common pond turtle that is often sold in the pet trade. The introduction of red-eared sliders has adversely affected populations of other species around the globe, as they are strong competitors and have shown an ability to reduce survivorship of other turtle species (Cadi and Joly, 2004; Chen and Lue, 1998; Luiselli et al., 1997). *Trachemys s. elegans* has proven to be a remarkably successful invader worldwide, having been found in ten U.S. states and 21 countries outside their

native range (Moll and Moll, 2004). The colonizing and competitive abilities of the red-eared slider have led to its inclusion in the IUCN list of the world's 100 most problematic invasive species (Lowe et al., 2000). They are not considered historically native to the Big Bend area, but have been introduced, most likely by people releasing their pet turtles when they no longer choose to care for them (Dixon, 2002; Spinks et al., 2003). Some evidence for westward range expansion of native Texas *T. s. elegans* has also been shown (Forstner et al., (in press)). So while there are native Texas *T. s. elegans* in the Pecos River and the southern Rio Grande valley of Texas, those native individuals did not historically disperse upstream on the Rio Grande beyond the Sanderson County line (Forstner et al., 1999). While historically most attention has been focused on introduction of species from distant lands and competitive interactions among them, more recently emphasis has been placed on the dangers of reshuffling of "native" species to areas outside of their native range, which can bypass isolation of closely related species and lead to biotic homogenization through hybridization (Perry et al., 2002). This is a serious threat, as red-eared sliders may not only compete for food and other resources with the Big Bend slider, but, since they are very closely evolutionarily related (Jackson et al., 2008), are purported to produce hybrid offspring. Hybridization may lead to loss of genetic diversity in *T. g. gaigeae*, and possibly to introgression. This is a particularly unique danger for turtles, which have already been shown to possess lower levels of genetic diversity than other organisms (Avise et al., 1992). In addition, these processes

can contribute to the loss of a species, especially a rare taxon (Rhymer and Simberloff, 1996). It has been suggested that hybridization between *T. g. gaigeae* and *T. scripta* in New Mexico only occurs where *T. s. elegans* is introduced (Degenhardt et al., 1996), and this has been suggested to occur in Big Bend National Park as well (Forstner et al., *in press*). In this study, the objectives were to assess the prevalence of hybridization in extant *T. g. gaigeae* populations, and determine whether hybridization is a result of introduction of red-eared sliders from distant populations or from westward range expansion of native *T. s. elegans*.

MATERIALS AND METHODS

Blood samples were collected from wild caught, pet trade, and zoo animals. Specific sampling was conducted from 1998-2008 within the range of *T. g. gaigeae* for both species as well as for potential hybrid individuals using baited hoop nets. Individuals were identified in the field using accepted morphological characteristics for each species, and an attempt was made to identify hybrids within the range of *T. g. gaigeae* by noting intermediacy of color pattern and markings between those expected for either full species (Seidel et al., 1999; Stuart, 1995). Blood was isolated from each individual and stored in Blood Storage Buffer (100 mM Tris pH 8.0, 100 mM Na_2EDTA, 10 mM NaCl, and 1% SDS) until needed. In addition, tissue samples were collected from individuals found dead, and stored in 70% ethanol at -20C. Remaining blood and/or DNA samples are in the MRJ Forstner frozen tissue collection at Texas State

University-San Marcos. DNA extraction from blood and tissue samples was conducted using a Biomek® 3000 Laboratory Automation Workstation (Beckman Coulter, Brea, CA), and a Wizard® SV 96 Genomic DNA Purification System (Promega, Madison, WI). DNA extractions were verified by agarose gel electrophoresis, and DNA visualized under UV light after ethidium bromide staining. The primers ND4 and Leucine used in PCR amplification were those described in Arévalo et al., (1994) because they show a high degree of conservation within turtles and were shown to be phylogenetically informative in reptiles (Arevalo et al., 1994; Forstner et al., 1995). Seven hundred base pairs of the ND4 mitochondrial gene were sequenced for 190 individuals (116 *T. g. gaigeae*, 74 *T. s. elegans*) using the aforementioned primers by the ICMB core DNA facility at the University of Texas-Austin. All sequences were aligned using Geneious 5.0 (Drummond et al., 2009). The software TCS (Clement et al., 2000) was used to produce a haplotype network using the statistical parsimony method (Templeton et al., 1992). *T. g. gaigeae* individuals possessing a haplotype from *T. s. elegans* were considered to be of hybrid origin.

Thirteen polymorphic microsatellite loci were amplified in 192 individuals for analysis of hybridization: 56 *T. s. elegans* collected from within the range of *T. g. gaigeae* as well as from other areas of the Rio Grande and Pecos rivers, 131 *T. g. gaigeae* from throughout its extant range, and 6 individuals identified as hybrids in the field. Nine loci (Gmu A19, B08, B21, D28, D55, D70, D87, D93, and D121) had been shown to amplify successfully in multiple emydid genera,

including *Trachemys scripta* (King and Julian, 2004). The other four loci were previously unpublished loci developed by Forstner and Davis for use in emydid turtles: MT3 (f GCTGCACAGAGTTACTTGGCAAG, r ACCCATCCATTCTGA CAATAGCTC), Tufu-2 (f TGCTCCTCATTATGGTACAGGGTG, r TCTGCCTCT CACACACAAACTCAG), Pseud 4-128 (f GCAAGGCTGCACAAACTCTC, r GCA GGTGTCCACATTGAC), and Pseud 225-2(f GCTTCTATGAAGATGGCT TTTTGAAC, r CCGCAGCATAC TAATTGACTTTG). Microsatellite PCR products were analyzed using fluorescently labeled primers and a Beckman CEQ 8800. F_{ST} values were calculated for each locus using GDA 1.1 (Lewis and Zaykin, 2001), based on the K value resulting from the structure analysis to ensure that the results were not due to the influence of only one or a few of the loci. These data were first analyzed using STRUCTURE (Pritchard et al., 2000) using models with and without admixture to both determine the ability of the loci to distinguish the two species as well as make an initial assessment of hybridization in the data. Both models were run for values of K (the number of clusters) from 1 through 15, with a burn-in of 100,000 generations followed by 500,000 generations for 5 independent runs at each value of K. The methods of Evanno et al. (2005) were used to determine the appropriate value for K. Individuals with a highest assignment probability of ≥ 0.80 were considered as unambiguously belonging to the respective group, those whose highest assignment probability was below this threshold were considered to be of admixed origin. NewHybrids 1.1 (Anderson and Thompson, 2002) was used to identify hybrids in the

microsatellite data set using a Jeffrey's like prior for both mixing proportions and allele frequency.

In an attempt to determine the origin and contribution of introduced individuals to hybridization, a data set (n=166) comprised of hybrids identified in the aforementioned analyses combined with data from *T. s. elegans* samples available from Texas, New Mexico, Oklahoma, Louisiana, Georgia, and Florida genotyped at the same 13 loci was analyzed using TESS 2.3 (Chen et al., 2007) which employs a Bayesian assignment process that includes spatial data to determine the number of populations or clusters (*K*) as well as to assign individuals to populations/clusters. To determine *K*, 100 independent runs of 50,000 iterations with a burn in of 20,000 iterations were conducted for values of *K* from 2-14. The CAR admixture model (Durand et al., 2009) was used as parental populations were of interest, and the spatial interaction parameter was estimated by the MCMC algorithm. The best value of *K* was chosen based on DIC (Deviance Information Criterion) and direct inspection of the output as described by the authors. Population assignments/admixture coefficients were calculated by averaging the coefficients from the 20% of the runs with the lowest DIC using CLUMPP (Jakobsson and Rosenberg, 2007), and ancestry was judged using the afforementioned threshold used in the STRUCTURE analyses.

RESULTS

mtDNA: Only 2 haplotypes (A and B) were recovered in *T. g. gaigeae* (Figure 1), with only 2 individuals possessing the second, both from the same sampling site (Black Gap Wildlife Management Area, TX). Seven haplotypes (C to J) were found in *T. s. elegans* (Figure1). Overall, mitochondrial sequence diversity was found to be very low, with haplotype groups for both species separated by only 7 substitutions (Figure 1). Of the individuals assessed in the field to be *T. g. gaigeae*, 109 had haplotype A and two haplotype B. Fifty-five individuals identified as *T. s. elegans* were confirmed as such by their haplotypes. Five individuals identified as *T. g. gaigeae* were found to have a *T. s. elegans* haplotype (3 from New Mexico (21513, 23869, 26865) and 2 from Big Bend National Park (19394, 19186)), and four individuals identified as hybrids in the field had *T. g. gaigeae* (2098, 2157, 2162, 1898) haplotypes. Eighteen individuals identified as *T. s. elegans* in the field were found to have *T. g. gaigeae* haplotypes (2040, 2041, 5872, 5873, 5880, 6186, 8171, 9803, 9886, 17546, 17961, 18884, 19178, 27599, 27601-4). Of these, 7 were from Big Bend National Park, 1 from Tamaulipas, Mexico, 5 from the Pecos River, one from the Devil's River, 2 from upstream of Amistad Reservoir in the area of Langtry, TX, 1 from Concho Co., TX, and one from the LBJ National Grassland west of the Dallas/Fort Worth area.

Microsatellite Analysis: The value of K selected under the STRUCTURE analyses was 2, corresponding to the two parental species, with some evidence of

hybridization for some individuals (Table 1). Locus-by-locus F_{ST} values calculated using this hierarchical arrangement show little evidence of the analyses following this arrangement being biased by dependence on a minority of the loci used (Table 2). One hundred sixteen individuals identified in the field as *T. g. gaigeae* were identified as such by NewHybrids, and 40 identified as *T. s. elegans* were likewise confirmed by NewHybrids. Analysis of microsatellite data with NewHybrids assigned 16 individuals to hybrid classes (Table 1). Five of these were identified as *T. g. gaigeae* in the field (2161, 2163, 18866, 19202, 21513), one was identified as a hybrid in the field (18854), and 10 individuals had been identified as *T. s. elegans* in the field (2040, 2041, 2316, 5872, 5873, 6186, 19178, 20734, 27603, 27605). Four individuals identified as hybrids in the field were assigned as *T. g. gaigeae* (2098, 2157, 2162, 18898). Six individuals identified as *T. s. elegans* in the field were assigned to *T. g. gaigeae* (9712, 9803, 9886, 9988, 10003, 17961). Ten individuals identified in the field as *T. g. gaigeae* were identified as *T. s. elegans* (18859, 18900, 18929, 19061, 19089, 19208, 19394, 19570, 21512, 21514; Table 1).

The TESS analysis to examine the origins of *T. s. elegans* and hybrids within the range of *T. g. gaigeae* determined 4 clusters or potential parental populations (*K*=4) (Figure 3). The first of the resulting putative parental populations appeared to represent *T. s. elegans* populations in eastern and central Texas through the Trans-Pecos region, and included one individual from a commercial turtle farm. The second population was composed of individuals

from northeast Texas, a Louisiana turtle farm, Georgia and Florida. The third population was predominantly composed of individuals whose ancestry had come into question in previous analyses. The fourth population appears to include the "native" southern Rio Grande *T. s. elegans* from the mouth of the Rio Grande in South Texas through Lake Amistad, and also includes populations in the upper Colorado, Nueces and Pecos rivers. Thirty-eight of the 40 individuals included that appeared to be of questionable ancestry previously were unambiguously assigned to one of these populations by the TESS analysis. None of these individuals was assigned to population 2. Twenty-two were assigned to population 4, 11 to population 3, and 5 to population 1 (Table 1).

DISCUSSION

The methods employed in this study clearly demonstrate that significant hybridization between *T. g. gaigeae* and *T. s. elegans* is occurring. It has also effectively demonstrated that attempts to detect hybrid origin of offspring using morphological characteristics is of little or no utility, as only one such individual was confirmed as a hybrid by molecular methods. The results of assessment with mtDNA were particularly interesting. Mitchondrial DNA proves to be of little utility in hybrid assessment between these taxa, as hybrids can only be identified in one direction (individuals identified morphologically as *T. g. gaigeae* had an *T. s. elegans* haplotype). This is due to the encapsulation of the diversity (or lack thereof) within the diversity of *T. s. elegans* (the predominant *T. g. gaigeae* haplotype A was found in *T. s. elegans*). This occurred with 9 individuals from

within a possible hybrid zone, 6 individuals from Rio Grande tributaries, 1 from Mexico, 1 from near San Angelo and 1 from the LBJ National Grassland near D/FW. This illustrates ancestral polymorphism or incomplete lineage sorting, as the Pecos and Devil's rivers were once more naturally connected with the Rio Grande, and these populations at one time likely experienced some gene flow. The appearance of this haplotype in the other two areas that were not historically connected to the Rio Grande is likely to be an artifact of human shuffling of turtles among populations, as both samples were collected near public access areas, which are the most common areas pet owners release unwanted animals. The low diversity found in mtDNA sequences in this study is not uncommon in studies of emydid turtles (Rosenbaum et al., 2007; Velo-Anton et al., 2008; Wiens et al., 2010), and thus could be considered conservative such that any divergence is representative of real divergence. The appearance of ancestral polymorphism in this study is typical of the recurring difficulties encountered in attempts to elucidate the evolutionary history of many emydid groups, especially of the genus *Trachemys*. It should be noted that, if many individuals from populations outside any expected hybrid zone had not been included (as in many studies), this feature would not have been uncovered and thus could lead to incorrect inferences in some cases.

Analysis of the microsatellite data appeared to be more efficient than the mtDNA data at detecting hybrids. It also demonstrates that most hybrids are cryptic in nature, as only one individual identified as hybrid in the field was

corroborated as hybrid by the microsatellite analysis. It also demonstrates the difficulty inherent in dealing with taxa that are so closely related. Individuals from the Pecos and Devil's rivers that exhibited ancestral polymorphism in the mtDNA analyses were assigned by NewHybrids to either hybrid or *T. g. gaigeae* genotypic groups. This illustrates that nuclear DNA (nDNA) in these individuals is also very similar to *T. g. gaigeae*, such that it brings the possibility that either they share ancestry with individuals that were introduced from another region, or the ancestral polymorphism is a result of former contact between these currently very distant populations. It has been clearly demonstrated in analyses using additional mtDNA sequence data (Jackson et al., 2008) as well as nDNA and combinations of both data types (Wiens et al., 2010) resolve the two taxa as distinct. It is also interesting to note that 5 individuals collected from the same area of the Pecos River are assigned to the expected genotypic class (*T. s. elegans*) demonstrating that there is a component of the population that is genotypically similar to other *T. s. elegans* as would be expected.

The TESS analysis recovered a clear southeastern population of red-eared sliders, and the fact that none of the individuals identified as hybrids by previous analyses were assigned to this population rejects the hypothesis that introduced individuals originated from the southeast. The composition of the third population is predominantly individuals identified as hybrids, and members of this population are geographically scattered demonstrative of the shuffling of turtles among populations by humans. That most of the hybrids are assigned to

population 4 lends credibility to the hypothesis that *T. s. elegans* native to the southern Rio Grande may have expanded their range, possibly due to the creation of Amistad reservoir. That this genotype is represented far up the Pecos is either a result of translocation of turtles or represents historical gene flow. Population 1 appears to be geographically centralized in central and east Texas (Figure 3), but includes individuals from the Rio Grande and Oklahoma, again suggesting potential translocation or spectacularly dramatic migration events. The assignment of hybrids to three of the four populations identified lends additional support to the importance of translocation of turtles to the production of hybrid offspring. It is possible that more extensive sampling of *T. s. elegans* would lead to a more resolved conclusion using the same methods, as it is possible that there is genetic structure present that was missed in this study, especially outside of Texas. It is also possible that translocation of animals has occurred sufficiently such that full resolution will elude us.

The occurrence of hybridization as observed in this study is a real threat to the genetic integrity of the Rio Grande endemic *T. g. gaigeae*. This alone justifies extensive *T. s. elegans* removal efforts within the historical range of *T. g. gaigeae*. Educational and enforcement efforts to discourage the release of captive *T. s. elegans* should also be encouraged. The results of this study with regard to the identity of individuals from the Langtry area suggest that there is some potential for a hybrid zone to exist in this area, and this deserves further study to elucidate the relationship between native Rio Grande *T. s. elegans* and

T. g. gaigeae distribution in the main stem of the Rio Grande River above Amistad reservoir.

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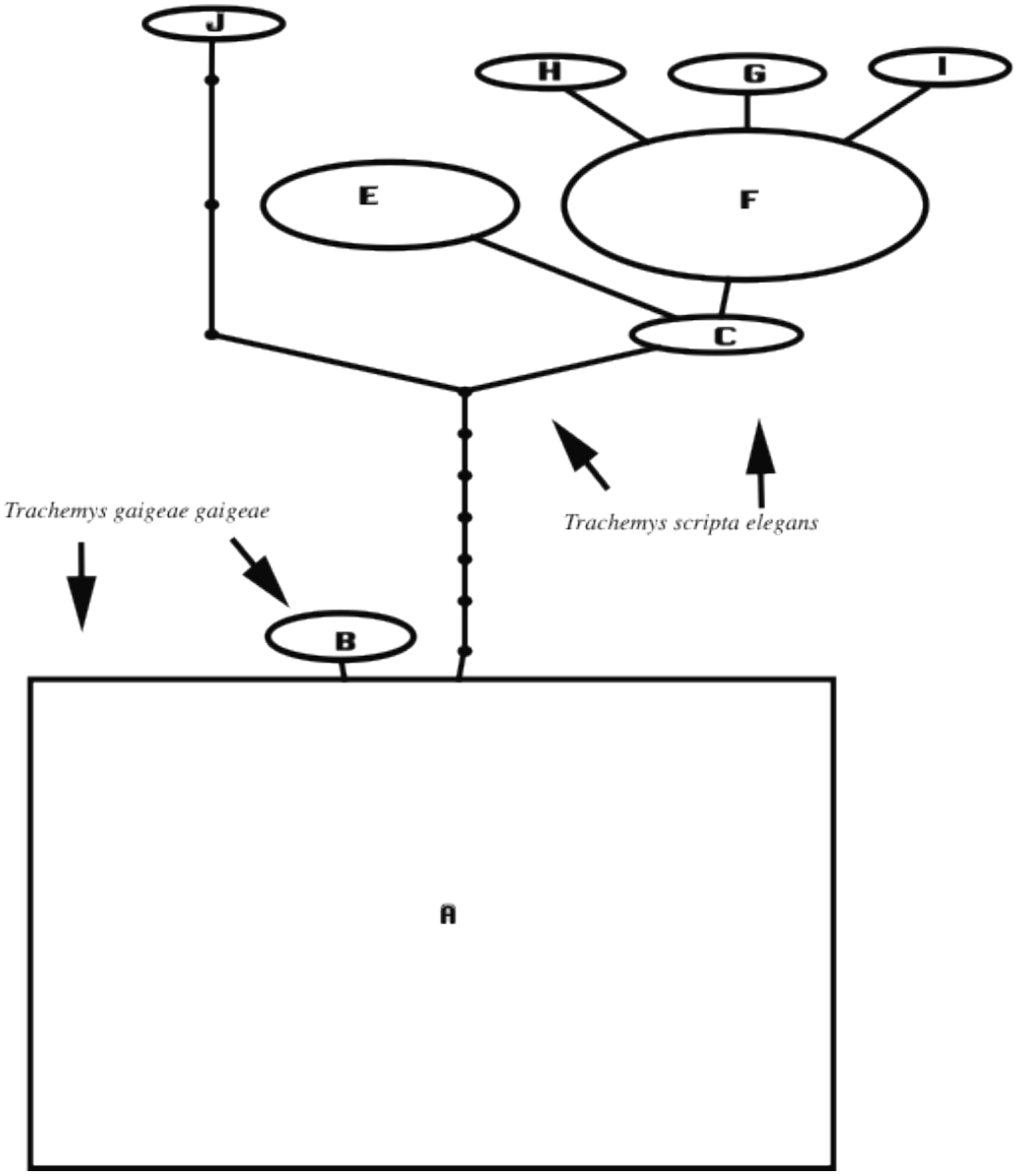

Figure 1. Haplotype network. Haplotype network constructed from 700 bp ND4 fragments from 172 indiviuals in this study. A (n=135) and B (n=2) are *T. g. gaigeae* haplotypes. C (n=5), E (n=17), F (n=30), G (n=3), H (n=2), I (n=2), and J (n=1) are *T. s. elegans*. Each line segment represents one nucleotide change (note that there are only 8 between the species). Haplotypes C, E, and F are found predominantly in south Texas, and F is found in Fla., Ga., Ok, and the Trans-Pecos region of Texas. Three hybrids were found with E and 6 with F. H was found only in the Pecos River. Haplotype I was only found in hybrids in New Mexico, and G was found in Fla. And Ga. Haplotype J was found in Fla.

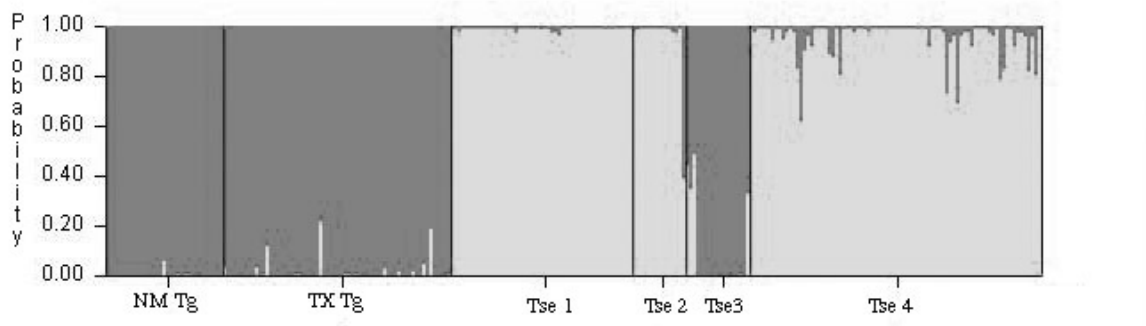

Figure 2. STRUCTURE analysis assignment probabilities. Assignment probabilities of individuals resulting from STRUCTURE analysis of microsatellite data at K=2. Darker shading indicates assignment to *Trachemys g. gaigeae*, lighter to *Trachemys scripta elegans*. Population labels on the x axis correspond to populations observed in analysis of the microsatellite data using TESS.

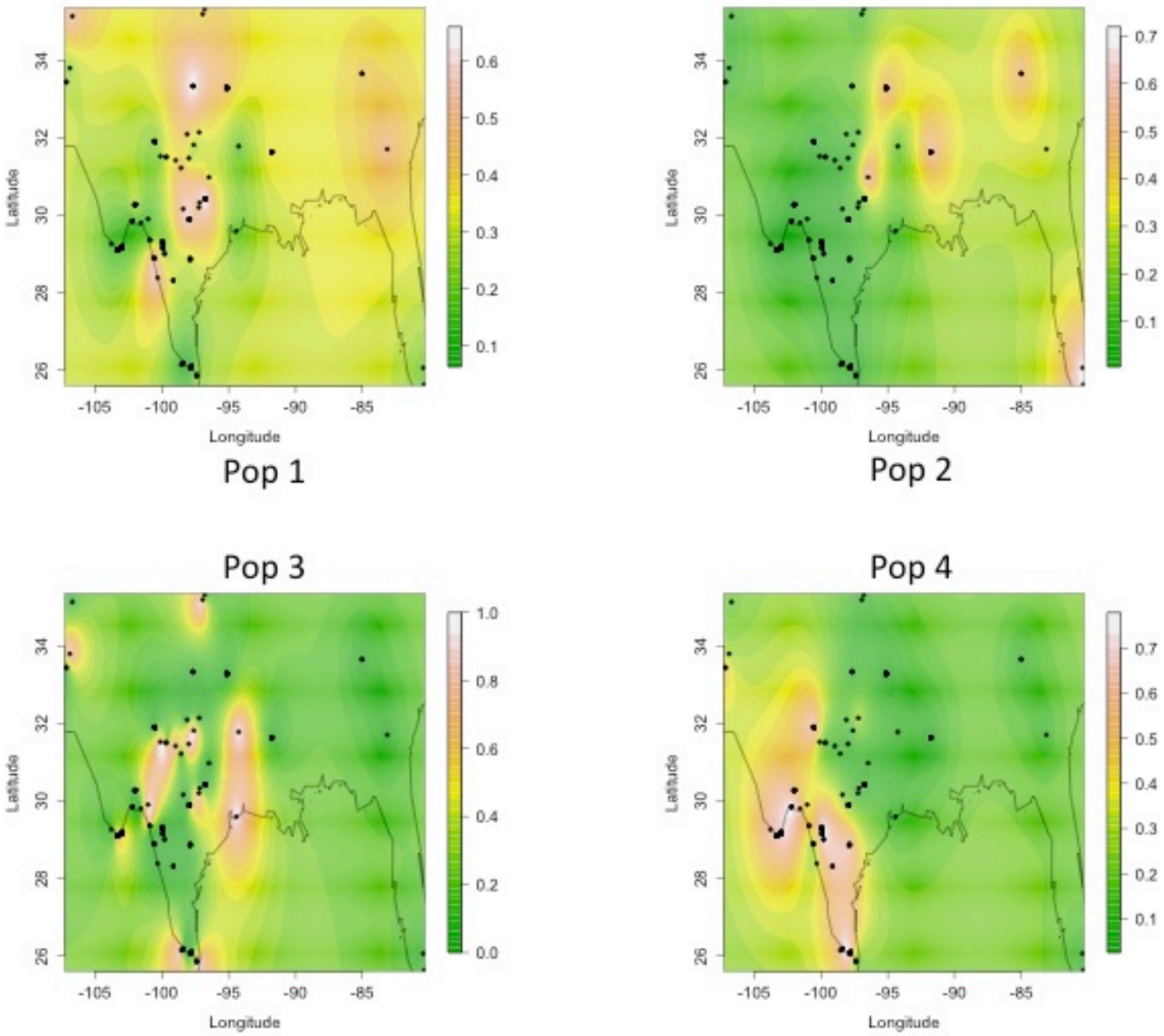

Figure 3. Spatial prediction of admixture proportions of *T. s. elegans* populations. Spatial representation of estimated admixture proportions of the 4 *T. s. elegans* populations resulting from the analysis of 13 polymorphic microsatellite loci with TESS 2.3. Population 1 is largely representative of central Texas individuals. Population 2 illustrates a southeastern population. Population 3 is composed mostly of individuals identified as *T. s. elegans* and *T. g. gaigeae* hybrids by other analyses. Population 4 represents "native" south Texas Rio Grande, Pecos and Nueces River *T. s. elegans*. Populations 1 and 3 show the best evidence of translocation of individuals. The largest number (22) of the hybrids were assigned to population 4. No hybrids were assigned to population 2.

Table 1. Assignment of Hybrid Individuals by Genotype and Haplotype. Specific assignment of problematic individuals according to different analysis methods. A dash represents missing data, TSE refers to *Trachemys scripta elegans*, TG refers to *Trachemys gaigeae*.

ID	Locality Descrption	Field ID	Structure (No admixture)	Structure (Admixture)	NewHybrids	Haplotype ID	TESS Pop assignment
2040	Langtry, TX	TSE	TSE	TSE	Hybrid	gaigeae	4
2041	Langtry, TX	TSE	TSE	Hybrid	Hybrid	gaigeae	4
2098	Big Bend Ranch, TX	Hybrid	-	-	TG	gaigeae	-
2157	Elelphant Butte Reservoir, NM	Hybrid	-	-	TG	gaigeae	-
2161	Albuquerque Nature Center, NM	TG	TSE	Hybrid	Hybrid	gaigeae	3
2162	Bosque Del Apache NWR, NM	Hybrid	-	-	TG	-	-
2163	Bosque Del Apache NWR, NM	TG	TG	Hybrid	Hybrid	gaigeae	3
2316	Langtry, TX	TSE	TSE	TSE	Hybrid	scripta	4
5872	Oasis Ranch, TX	TSE	TSE	TSE	Hybrid	gaigeae	4
5873	Oasis Ranch, TX	TSE	TSE	TSE	Hybrid	gaigeae	4
5880	Oasis Ranch, TX	TSE	TSE	TSE	TSE	gaigeae	4
6186	Oasis Ranch, TX	TSE	TSE	TSE	Hybrid	gaigeae	4
8171	LBJ Ntl Grassland, TX	TSE	TSE	TSE	-	gaigeae	1
9712	Del Rio, TX	TSE	TG	TG	TG	-	3
9803	Oasis Ranch, TX	TSE	TG	TG	TG	gaigeae	3
9886	Dolan Falls, TX	TSE	TG	TG	TG	gaigeae	3
9988	Southmost, TX	TSE	TG	TG	TG	-	3
10003	Southmost, TX	TSE	TG	TG	TG	-	3
17546	Haechton, TX	TSE	TG	TG	-	gaigeae	3
17961	Lake Gomez, Tamaulipas, Mexico	TSE	TG	TG	TG	gaigeae	3
18854	Big Bend National Park, TX	Hybrid	-	-	Hybrid	gaigeae	-
18859	Big Bend National Park, TX	TG	TSE	TSE	TSE	-	1
18866	Big Bend National Park, TX	TG	TG	Hybrid	Hybrid	-	3
18884	Big Bend National Park, TX	TSE	TSE	TSE	TSE	gaigeae	4
18898	Big Bend National Park, TX	Hybrid	-	-	TG	gaigeae	-
18900	Big Bend National Park, TX	TG	TSE	TSE	TSE	-	1
18929	Big Bend National Park, TX	TG	TSE	TSE	TSE	-	4
19061	Big Bend National Park, TX	TG	TSE	TSE	TSE	-	1
19089	Big Bend National Park, TX	TG	TSE	TSE	TSE	-	4
19178	Big Bend National Park, TX	TSE	TSE	Hybrid	Hybrid	gaigeae	4
19186	Big Bend National Park, TX	TG	TG	TG	TG	scripta	3
19202	Big Bend National Park, TX	TG	TSE	Hybrid	Hybrid	-	4
19208	Big Bend National Park, TX	TG	TSE	TSE	TSE	gaigeae	4
19394	Big Bend National Park, TX	TG	TSE	TSE	TSE	scripta	4
19570	Big Bend National Park, TX	TG	TSE	TSE	TSE	-	1
20734	Lajitas, TX	TSE	TSE	TSE	Hybrid	-	4
21512	Elelphant Butte Reservoir, NM	TG	TSE	TSE	TSE	-	4
21513	Elelphant Butte Reservoir, NM	TG	TSE	TSE	Hybrid	scripta	1
21514	Elelphant Butte Reservoir, NM	TG	TSE	TSE	TSE	gaigeae	4
23869	Ft Craig, NM	TG	-	-	-	scripta	-
26865	Elelphant Butte Reservoir, NM	TG	-	-	-	scripta	-
27599	Big Bend National Park, TX	TSE	TSE	TSE	TSE	gaigeae	4
27601	Big Bend National Park, TX	TSE	TSE	TSE	TSE	gaigeae	4
27602	Big Bend National Park, TX	TSE	TSE	TSE	TSE	gaigeae	4
27603	Big Bend National Park, TX	TSE	TSE	TSE	Hybrid	gaigeae	4
27604	Big Bend National Park, TX	TSE	TSE	TSE	TSE	gaigeae	4
27605	Big Bend National Park, TX	TSE	TSE	TSE	Hybrid	-	4

Table 2. Locus-by-Locus F_{ST} Values for Microsatellite Loci. Locus-by-locus F_{ST} values for the thirteen microsatellite loci used in analysis of hybridization between *Trachemys gaigeae* and *Trachemys scripta elegans* calculated using the hierarchy resulting from analysis of the data using STRUCTURE. All loci show non-trivial influence on the separation of the data into these two groups.

Locus	*MT3*	*TUFU2*	*P4128*	*P225*	*A19*	*B08*	*D70*	*B21*	*D28*	*D55*	*D87*	*D93*	*D121*
F_{ST}	0.19	0.09	0.38	0.48	0.17	0.14	0.08	0.06	0.09	0.32	0.3	0.34	0.09

CHAPTER IV

POPULATION GENETICS OF *TRACHEMYS GAIGEAE GAIGEAE* AND COMPARISON TO POPULATIONS OF *TRACHEMYS SCRIPTA ELEGANS*

The Big Bend slider (*Trachemys gaigeae gaigeae*) is an emydid turtle that was once common throughout the Rio Grande River from west Texas into New Mexico. This turtle is a unique lineage of slider (Jackson et al., 2008) with a small geographic distribution (Ernst and Lovich, 2009; Stuart and Ward, 2009), in compromised habitat and, after evaluation by the World Conservation Union (IUCN), was placed on the Red List as a species vulnerable to extinction due to range contraction and loss of habitat (Baillie and Groombridge, 1996). Because of the impacts to the watershed, the distribution of this species has been undergoing fragmentation for some time (Ernst et al., 1994). In fact, the river that comprises most of the species remaining habitat, the Rio Grande, is threatened to the extent that it was named one of the world's top ten rivers at risk (Wong et al., 2007) largely due to anthropogenic impacts. Currently, there are several large gaps in the distribution of the species along this section of the river. Surveys in the late 1990's documented that *T .g. gaigeae* has been extirpated from the larger portion of its historic distribution, with remaining populations

centered in the Big Bend region of Texas (Forstner et al., (in press); Forstner et al., 1999). The largest extant population in the USA is generally contained withinthe boundaries of Big Bend National Park, which also represents the largest contiguous area of *T. g. gaigeae* habitat that is under protection. Smaller populations are scattered from Elephant Butte reservoir to the Bosque del Apache National Wildlife Refuge in New Mexico, and most recently, a small remnant population was detected in Hudspeth County, Texas (Figure 1), which lies in between the Big Bend and Elephant Butte populations (Forstner et al., (in press)). Virtually no recent data exist for the evaluation of the species in the Rio Conchos in Mexico (the major tributary of the Rio Grande upstream of Big Bend National Park near Presidio). This tributary is assumed to have held a similarly abundant population, but is also likely to have suffered the same consequences resulting from anthropogenic pressures such as dam construction, flow reduction and channelization. The long-term consequences of these changes to the river on the watershed as an ecosystem are not clear, nor have the impacts to Rio Grande endemics like the Big Bend slider, been quantified. If enough of the habitat is lost or made unsuitable, historically contiguous populations can become fragmented and isolated, resulting in reduced or interrupted gene flow with attendant losses to genetic diversity (Gerlach and Musolf, 2000).

T. g. gaigeae now faces an additional threat from red-eared sliders, *Trachemys scripta elegans* (see chapter 3). *Trachemys s. elegans* is the sister taxon to *T. g. gaigeae* (Jackson et al., 2008; Wiens et al., 2010). Despite the

breadth of scientific study of *T. s. elegans* (Ernst and Lovich, 2009; Gibbons, 1990) relatively few population genetics analyses for that species are available to provide a comparative context with *T. g. gaigeae*. We have included in our analyses comparative evaluation of *T. s. elegans* as part of our assessment of the hybridization of these two taxa (see chapter 3). While presumptive, no one has implied that *T. s. elegans* is in decline among drainages in the USA and consequently we sought to evaluate the comparative population genetics in a taxon occurring both within the compromised Rio Grande and outside it.

In this study, we sought to evaluate the current status of extant *T. g. gaigeae* populations using multiple polymorphic microsatellite markers. It has been shown that mitochondrial DNA in turtles evolves very slowly (Avise et al., 1992), making it of little utility for population level investigations in many populations, however non-coding nuclear DNA regions are thought to be much less conserved and therefore potentially much more useful when evaluating population characteristics of turtles (King and Julian, 2004).

Materials and Methods

Blood samples were collected from wild caught, pet trade, and zoo animals by various researchers. Specific sampling was conducted within the range of *T. g. gaigeae* from 1998-2008 for both species. Individuals were identified in the field using accepted morphological characteristics for each species (Ernst et al., 1994; Stuart and Ward, 2009). Blood was retrieved from

each individual and stored in Blood Storage Buffer (100 mM Tris pH 8.0, 100 mM Na_2EDTA, 10 mM NaCl, and 1% SDS) until needed. Tissue samples were collected from individuals found dead, stored in ethanol and frozen at -80°C. DNA extraction from blood and tissue samples was conducted using a Biomek® 3000 Laboratory Automation Workstation (Beckman Coulter), and a Wizard® SV 96 Genomic DNA Purification System. DNA extractions were verified by agarose gel electrophoresis and visualized by ethidium bromide under UV light. Remaining blood and/or DNA samples are stored in the MRJ Forstner frozen tissue collection at Texas State University-San Marcos.

Thirteen polymorphic microsatellite loci were amplified in 97 *T. g. gaigeae* individuals previously determined to be of pure ancestry. For comparative analysis 166 *T. s. elegans* collected from within the range of *T. g. gaigeae* as well as from other areas of the Rio Grande and Pecos rivers, Texas, New Mexico, Oklahoma, Louisiana, Georgia, and Florida were genotyped at the same 13 loci. The latter group included 40 individuals collected within areas of possible hybridization of the two species and identified as hybrids to avoid confounding the inference of true population structure in *T. g. gaigeae*. Nine of the thirteen loci (Gmu A19, B08, B21, D28, D55, D70, D87, D93, and D121) had been isolated in *Glyptemys muhlenbergii* and been shown to amplify successfully in multiple emydid genera, including *Trachemys scripta* (King and Julian, 2004). The other four loci were unpublished loci developed by Forstner and Davis for use in Emydid turtles: MT3 (f GCTGCACAGAGTTACTTGGCAAG,

rACCCATCCATTCTGACAATAGCTC), Tufu-2 (f TGCTCCTCATTATGGTACAGGGTG, r TCTGCCTCTCACACACAAACTCAG), Pseud 4-128 (f GCAAGGCTGCACAAACTCTC, r GCAGGTGTCCACATTGAC), and Pseud 225-2 (f GCTTCTATGAAGATGGCTTTTTGAAC, r CCGCAGCATACTAATTGACTTTG). PCR (polymerase chain reaction) was conducted using Promega *Taq* polymerase and standard protocols in 10 *µ*l reactions with annealing temperatures of 55-65°C. Microsatellite PCR products were analyzed using fluorescently labeled primers and a Beckman CEQ 8800. To examine the extent of isolation by distance (IBD) in each species, Mantel tests as implemented in Alleles In Space (Miller, 2005) were conducted on the microsatellite data for each species. To assess population structure and determine the number of true parental populations represented in the dataset for each species, the microsatellite data was analyzed using TESS 2.3 (Chen et al., 2007) which employs a Bayesian assignment process that includes spatial data to determine the number of populations (*K*) as well as assign individuals to populations. To determine *K*, 100 independent runs of 50,000 iterations with a burn in of 20,000 iterations were conducted for values of *K* from 2-14 for *T. s. elegans* and 2-10 for *T. g. gaigeae*. Analyses for each species were conducted using both models without admixture to investigate presence of discrete populations and the CAR admixture model (Durand et al., 2009) to examine the distribution of parental populations and admixture among them. The spatial interaction parameter was estimated by the MCMC algorithm and the best value

of *K* chosen based on DIC (Deviance Information Criterion) and direct inspection of the output as described by the authors. Population assignments/admixture coefficients were calculated by averaging the coefficients from the 20% of the runs at the appropriate values of *K* with the lowest DIC using CLUMPP (Jakobsson and Rosenberg, 2007). For each population resulting from the TESS analysis, exact tests for Hardy-Weinberg equilibrium (HWE) were performed using GDA 1.1 (CITATION) and corrected for multiple comparisons using the sequential Bonferroni method (Rice, 1989). Exact tests for linkage disequilibrium were also performed using the "preserve genotypes" method in GDA to remove the influence of deviations from HWE. Wright's F-statistics were computed across populations of each species using analysis of variance (Weir and Cockerham, 1984) as implemented in GDA. To evaluate the effect of potential null alleles on measures of population differentiation, pair-wise F_{ST} values were calculated with and without the ENA correction implemented in the software FreeNA (Chapuis and Estoup, 2007). *T. g. gaigeae* populations were tested for signatures of recent population bottlenecks using BOTTLENECK (Piry et al., 1999) to perform Wilcoxon's signed rank test under the infinite alleles model (IAM) and two-phase model (TPM) with 95% step-wise mutations following the recommendations of the authors for microsatellite loci, as well as employing the mode-shift method (Luikart et al., 1998). Effective population size (Ne) of *T. g. gaigeae* populations was estimated using the Bayesian approximation method

implemented in ONeSAMP (Tallmon et al., 2008) as well as the linkage disequilibrium method implemented in LDNE (Waples and Do, 2008).

RESULTS

A total of 97 *T. g. gaigeae* (33 from New Mexico, 64 from Texas) and 166 *T.s. elegans* were genotyped. The *T. s. elegans* group included 2 individuals from FL, 3 from GA, 6 from LA, 2 from OK, 6 from NM and 1 from Mexico. The other 146 individuals were from Texas, including 22 from the Nueces River, 9 from the Pecos River, 55 from the Rio Grande (28 within Big Bend National Park) and the remainder from available samples collected from streams, small water bodies and roadways across the state. The average number of alleles per locus was 5.42 across *T. g. gaigeae* populations and 12.15 across *T. s. elegans* populations (Table 1). Both species exhibited high inbreeding coefficient values (F_{IS}) and moderate levels of population differentiation (F_{ST}) (Table 1), with populations of *T. g. gaigeae* being more divergent overall than those of *T. s. elegans*. Uncorrected pair-wise F_{ST} values did not differ strongly from those generated using the ENA correction for null alleles (Table 2).

Moderate but significant correlation between geographic and genetic distance was detected by the Mantel tests of the microsatellite data for both *T. g. gaigeae* (r=0.153, p=0.0009) and *T. s. elegans* (r=0.185, p=0.0009). The results of the TESS analysis of *K* were generally congruent across models with and

without admixture, providing evidence of 2 populations, New Mexico and Texas, in *T. g. gaigeae* (Figure 1) and 4 populations in *T. s. elegans* (Figure 2). The difference between the results of models with and without admixture in *T. g. gaigeae* were in regards to different assignments of individuals of admixed ancestry that provide some evidence of a cline in allele frequency between the two populations. Ten individuals captured in Texas were assigned to the New Mexico population by the model without admixture, but were ambiguously ($q<0.9$) assigned to both populations under the admixture model. Six individuals captured in Texas between the inflow of the Rio Conchos at Presidio and the New Mexico state line were assigned to the New Mexico population by both models, and 4 individuals also captured in Texas were assigned to the New Mexico population by the model without admixture, however these 4 were unambiguously assigned ($p>0.89$) to the Texas population by the admixture model. No significant departures from Hardy-Weinberg equilibrium were found in any population after sequential Bonferroni correction was applied. Of 468 total pair-wise tests (156 *T. g. gaigeae*, 312 *T. s. elegans*) for linkage disequilibrium, 35 showed significant linkage (11 *T. g. gaigeae*, 24 *T. s. elegans*). Only 8 of these occurred in more than one population, none occurred in more than 2 of the 6 total populations, and for those that occurred in 2 populations one population was of each species. The irregular distribution of significant results for linkage disequilibrium suggests that it is unlikely any true significant linkage exists.

Expected heterozygosity was higher than that observed for all populations of both species.

Population 5, a population of *T. s. elegans* composed mostly of individuals determined to be hybrids in a previous study (Previous Chapter), was much less divergent from *T. g. gaigeae* populations than other *T. s. elegans* populations, as would be expected if admixture between the two species was present, and was the most divergent among all *T. s. elegans* populations. No signature of a recent bottleneck was found in either *T. g. gaigeae* population, however the Texas population (population 2) provided a significant result (p=0.002) from the Wilcoxon's test for heterozygote deficiency under the TPM model only. Estimates of effective population size (N_e) from the Bayesian approximation were 27.64 (95% CI=17.25-73.38) for the New Mexico population and 77.75 (95% CI=50.9-235.98) for the Texas population. N_e estimates from the linkage disequilibrium method were congruent, 32.3 (95% CI=15.3-143.1) for the New Mexico population and 86.9 (95% CI=50.2-218) for the Texas population.

Discussion

While models with and without admixture resulted in the choice of the same value for *K* in analyses of each species, the admixture model generated the most biologically sensible conclusion as far as the individual assignments, especially considering that parental populations were of primary interest and that

there was an expectation of admixture due to translocation of individuals by humans, hybridization and potential for past gene flow. Therefore, only the individual assignments generated by the admixture models were considered. Significant results from Mantel tests for IBD were likely influenced by the relatively large geographic distances among populations recovered, as well as the presence of large geographic gaps in sampling that were a result of both the feasibility of such extensive sampling as well as the extirpation of *T. g. gaigeae* from large portions of its historic range (Ernst and Lovich, 2009; Forstner et al., (in press)). The presence of two divergent populations of *T. g. gaigeae* supports the idea of their extirpation in the middle portion of their range between the inflow of the Rio Conchos at Presidio, TX and Caballo reservoir, NM as suggested by Forstner (in press). With the exception of population 5, which was composed predominantly of individuals confirmed to be hybrids by a previous study, *T. s. elegans* populations are much less divergent from each other than the two *T. g. gaigeae* populations are from each other. The difference in assignments between the models with and without admixture appears likely to be the result of a cline in allele frequencies between the peripheries of the two populations that is a relic of historic gene flow between the two populations. Due to the small extent of this cline and the high degree of genetic conservation generally observed in emydid turtles combined with their long generation time, it is likely that this gene flow occurred a long time ago.

Diversity and heterozygosity are lower in *T. g. gaigeae* than their close relative, *T. s. elegans*. The high F_{IS} values and significant result of the sign test for heterozygote deficiency in *T. g. gaigeae* are also demonstrative of the specie's low diversity. While *T. s. elegans* appears to contain more diversity, it is a much more wide spread species. High genetic conservation has long been accepted as a well known feature of chelonian species (Avise et al., 1992; King and Julian, 2004). It is also instructive to note that the more widely distributed and successful *T. s. elegans* also exhibits high F_{is} values and Ho < He. This suggests that this type of results may be common for some turtle populations as a result of the order's intrinsically high genetic conservation (Avise et al., 1992; FitzSimmons et al., 1995; King and Julian, 2004). This combined with the failure to detect any signature of a bottleneck in *T. g. gaigeae* populations and the previously mentioned cline between the two populations suggests that *T. g. gaigeae* populations may have been small for a much longer period of time than previously assumed. Bottlenecks are often difficult to detect even when population reductions are known to have occurred (Busch et al., 2007), and most methods depend on the detection of excess heterozygosity during a fairly short window (Cornuet and Luikart, 1996; Piry et al., 1999), which may not be appropriate for turtle populations. In fact, given the high degree of conservation in turtles relative to other organisms, bottlenecks may be much more difficult to detect. It is also likely that the genetic structure and diversity of *T. g. gaigeae* has been impacted by human mediated translocation of turtles, including release of

captive *T. s. elegans* and possibly even *T. g. gaigeae*, which may have been exacerbated by potential hybridization in captivity and the often cryptic nature of hybridization events (see chapter 3). Removal of *T. g. gaigeae* for private collections has also occurred, is presently still legal in certain instances, and is likely to have been exacerbated by the much higher value of rare and endemic species in the collector market. Harvest of *T. g. gaigeae* for consumption may have occurred, but most likely only in a localized sense as its small size would not likely make it competitive with other species in commercial markets. The impact of this type of interference with wild reptile and amphibian populations is difficult to determine, and is potentially severely underestimated (Schlaepfer et al., 2005).

Currently, *T. g. gaigeae* populations receive little effective protection (Stuart and Ward, 2009). Given the low genetic diversity, the homogeneous population structure, and the small effective and directly estimated population size of *T. g. gaigeae*, the conservation status of this species should be elevated to endangered or even critically endangered. The determination between those to extreme levels of vulnerability can only truly be assessed by evaluation of the Mexican populations of the taxon. This is especially true in light of information regarding threats from hybridization and introduction of turtles from other populations now documented to occur in both NM and TX (Previous Chapter), as well as continuing and increasing demands on the river that provides them with habitat (Wong et al., 2007).

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Table 1. Summary Statistics for *T.g.gaigeae* and *T. s. elegans* populations. Summary statistics for *T. g. gaigeae* and *T. s. elegans* populations utilized in this population genetic evaluation. Sample size (N), proportion of polymorphic loci (P), average alleles/locus (A), expected (He) and observed (Ho) heterozygosity, and F-statistics across 13 polymorphic microsatellite loci.

		N	P	A	He	Ho	F_{IS}	F_{IT}	F_{ST}
T.g.gaigeae	NM (1)	33	0.85	3.77	0.39	0.25	0.35		
	TX (2)	64	1.0	7.07	0.46	0.28	0.40		
	mean		0.92	5.42	0.43	0.27	0.38	0.49	0.18
T.s.elegans	3	51	1	16.85	0.86	0.63	0.26		
	4	15	1	10.3	0.86	0.60	0.32		
	5	18	1	6.23	0.57	0.43	0.25		
	6	82	1	15.23	0.82	0.59	0.29		
	mean		1	12.15	0.78	0.56	0.28	0.35	0.09

Table 2. Pair-Wise F_{ST} values. Pair-wise F_{ST} values calculated among *T. g. gaigeae* (1=NM, 2=TX) and *T. s. elegans* populations with ENA correction for null alleles (upper triangle) and without correction (lower triangle) illustrate that divergence estimates are not appreciably affected by presence of null alleles.

	1	*2*	*3*	*4*	*5*	*6*
1		0.147	0.293	0.344	0.081	0.255
2	0.178		0.263	0.295	0.093	0.230
3	0.316	0.295		0.031	0.197	0.038
4	0.366	0.326	0.037		0.223	0.050
5	0.091	0.102	0.208	0.229		0.169
6	0.274	0.260	0.044	0.056	0.178	

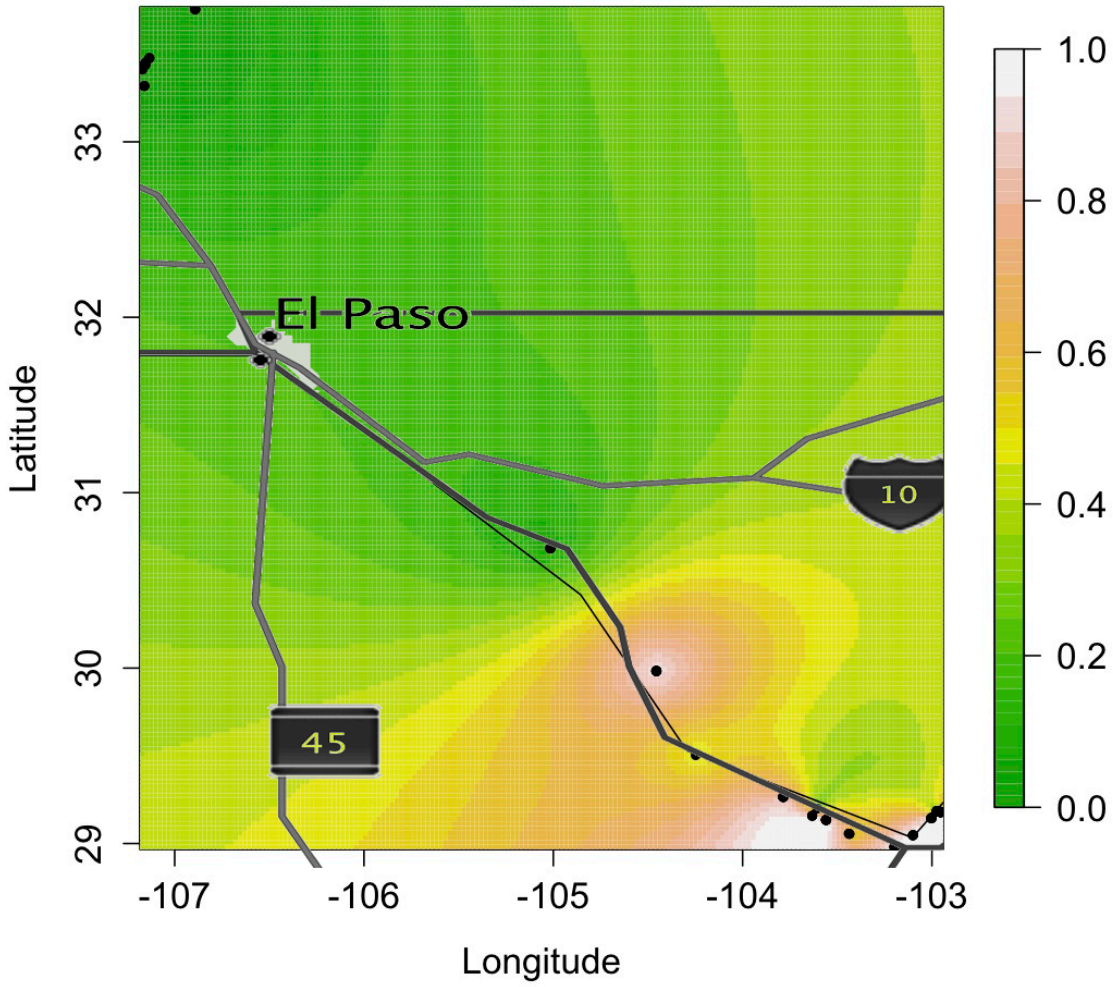

Figure 1. Admixture of extant *T. g. gaigeae.* Geographic prediction of admixture proportions for extant *T. g. gaigeae* populations from analysis of 13 polymorphic microsatellite loci using TESS 2.3. Legend shows colors representing admixture coefficients relative to the Texas *T. g. gaigeae* population for individuals by geographic location. The lightest shading represents 100 % genotypic membership to this cluster, while the darkest shading represents 0% membership. Points represent sampling locations. This illustrates the presence of individuals in the Texas population that share some ancestry with the New Mexican population, and the boundary of the two populations.

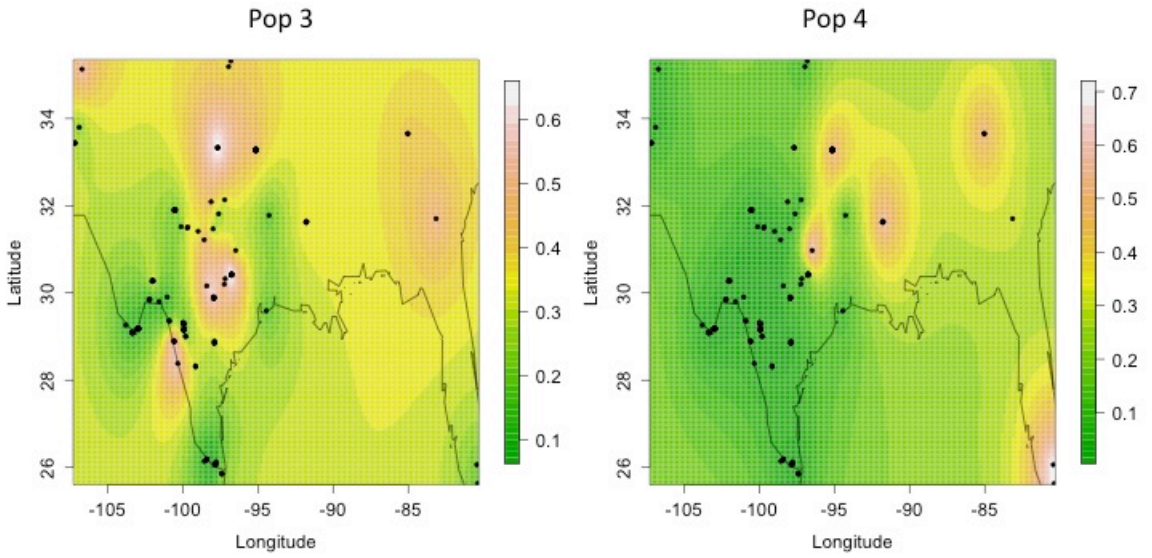

Figure 2. Spatial representation of admixture of 4 *T. s. elegans* populations. Spatial representation of estimated admixture proportions of the 4 *T. s. elegans* populations resulting from the analysis of 13 polymorphic microsatellite loci with TESS 2.3. Legend shows colors representing admixture coefficients relative to that population for individuals by geographic location. The lightest shading represents 100 % genotypic membership to this cluster, while the darkest shading represents 0% membership. Points represent sampling locations. Population 3 is largely representative of central Texas individuals and shows evidence of translocation of individuals. Population 4 illustrates a southeastern population, but the resolution is clearly affected by low sample size. However, the fact that no potential hybrid *T. g. gaigeae* X *T. s. elegans* individuals were shown to have ancestry in this cluster demonstrates that individuals introduced into the Rio Grande are unlikely to have originated from this geographic region, even though individuals from a commercial farm on the other side of the Mississippi River are assigned to this group.

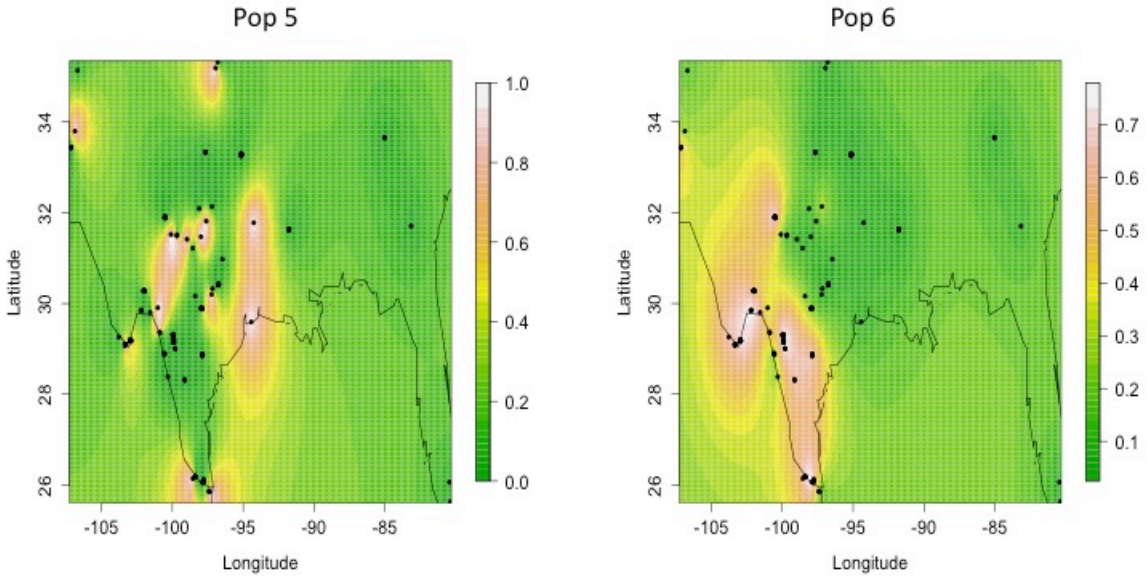

Figure 2 (continued). Spatial representation of estimated admixture proportions of the 4 *T. s. elegans* populations resulting from the analysis of 13 polymorphic microsatellite loci with TESS 2.3. Legend shows colors representing admixture coefficients relative to that population for individuals by geographic location. The lightest shading represents 100 % genotypic membership to this cluster, while the darkest shading represents 0% membership. Points represent sampling locations. Population 5 is composed mostly of individuals identified as *T. s. elegans* X *T. g. gaigeae* hybrids by other analyses and shows evidence of translocation of individuals. Population 6 represents "native" south Texas Rio Grande, Pecos and Nueces River *T. s. elegans*.

CHAPTER V

DEMOGRAPHY OF BIG BEND SLIDER POPULATIONS IN THE BIG BEND REGION OF TEXAS

The Big Bend slider (*Trachemys gaigeae gaigeae*), an emydid turtle that was once common throughout the Rio Grande from west Texas into New Mexico. This turtle is a unique lineage of North American slider (Jackson et al. 2008) with a small geographic distribution, in compromised habitat and, after evaluation by the World Conservation Union (IUCN) was placed on the Red List as a species vulnerable to extinction due to range contraction and loss of habitat (Baillie and Groombridge 1996). Several large gaps in their distribution between Sierra County (New Mexico) and Presidio County (Texas) may have existed for some time (Stuart and Ward 2009). Surveys in the late 1990's documented that *T. g. gaigeae* has been extirpated from the larger portion of its historic distribution, with the largest extant population centered around the vicinity of Big Bend National Park, which also represents the largest contiguous area of *T. g. gaigeae* habitat that is under protection (Forstner et al. 1999, Forstner et al. (in press)). Big Bend National Park encompasses a vast area containing great biodiversity bordered by the Rio Grande River to the south. This river is often the only water available for many miles and the river ecosystem itself is listed as one of the 10 rivers

most-at-risk worldwide due to overdraw of water (Wong et al. 2007). Given the climbing anthropogenic pressures on this watershed (especially on the unprotected portions of it), the fate of many Rio Grande endemic species may lie in the management of populations within these protected areas. In the case of *T. g. gaigeae*, however, very little information about aspects of their biology, ecology or populations has been published in contrast to their much-studied relative *T. scripta* (Ernst and Lovich 2009, Stuart and Ward 2009). The objectives of this study were to characterize the long-term demography of the population of *T. g. gaigeae* in the Big Bend region using capture-recapture techniques and survival analysis.

MATERIALS AND METHODS

Sampling. We chose 8 sampling sites spread across Big Bend National Park, and 1 site in Big Bend Ranch State Natural Area to the west of Lajitas, Texas (Figure 1). All sampling took place during the most active season for *T. g. gaigeae*, between late April and July from 2005-2009, for a total of 2,855 trap days over the 5-year period. Canoes were used to place baited hoop nets along approximately 2-5 km of the river at each site. Traps were checked at least once every 48 hours (on average once every 24 hours). Each individual capture was marked using marginal scute notching. Straight carapace length (CL) was measured from the nuchal notch to the pygal notch. Carapace width (CW) was recorded as the straight line measurement to the outsides of the marginal scutes across the center of the bridge. Plastron length (PL) was taken as the straight

line lengthwise measurement of the centerline of the plastron, and plastron width (PW) was recorded as the width between the outer sutures of the abdominal scutes of the plastron. Body depth (BD) was the greatest distance from the top of the carapace to the bottom of the plastron. All measurements were taken with calipers (Haglof, Sweden) to the nearest millimeter. Mass was taken to the nearest gram and photos from the dorsal and ventral side were taken prior to release. Sex was determined by size and shape of the tail and the relationship of the vent opening to the margin of the carapace. Sexual maturity, (henceforth, adult) status, was judged based on the CL measurements of 105 mm for males and 170 mm for females (Legler 1960). Recaptures were identified, CL measured and weighed, photographed from dorsal and ventral aspect to validate individual identity, and released. The identity of each recapture was confirmed by comparison of recapture photographs to photographs from previous encounters prior to data analyses. All capture and recapture locations, as well as trap sites, were recorded using a hand held GPS (Garmin GPSmap 60Cx).

Data analyses. We used the Cormack-Jolly-Seber (CJS) model as implemented in program MARK (White and Burnham 1999) to estimate apparent survivorship (φ) and probability of capture (p). Model selection was made based on Akaike Information Criterion (AIC, Akaike 1973) corrected for small sample size (AIC_c, Hurvich and Tsai 1989). We chose 16 candidate models, which allowed both φ and p to be constant, dynamic or to vary with sex and sex and time (Table 1). Population size (N) was estimated using the Jolly-Seber (JS)

method as modified in POPAN5 (Schwarz and Arnason 1996) and implemented in MARK, with the best model again chosen by AIC_c. This model process included the additional parameters N and "pent" (probability of entry). Phi and *p* were again allowed to be static or vary with time, sex, or time and sex, while pent was allowed to be static or vary with time and N was treated as static. Estimates of φ and *p* were compared among the best models selected in each case.

Average yearly growth in carapace length and weight for recaptured individuals was calculated across the greatest possible time interval to minimize error. The adult sex ratio was calculated from capture data and tested for differences from parity using a chi-square goodness of fit test, corrected for one degree of freedom.

RESULTS

A total of 445 individuals were captured (270 males and 175 females). Seventy-eight were recaptured once, and 12 were recaptured more than once throughout the study period. The maximum number of times an individual was recaptured was 3. We captured 371 adults (242 males and 129 females). The sex ratio was 1.88 males: 1 female, thus significantly male biased (x^2=34.42, $p<0.0001$). Average CL and weight reflected reported sexual dimorphism in the species (Ernst and Lovich 2009) (Table 2, Figure 2). Adult average yearly growth was greater for females than males (Table 3). Individual movements of up to ~64 miles (7 of 5.5 miles or less, 1 ~26 miles, 1 ~64 miles) were recorded over the

study period. Ninety percent of recaptured individuals were recaptured at their original capture site.

Based on ΔAIC_c scores, the best model in the CJS analysis suggested that φ (apparent survival) was static and *p* (capture probability) varied with time, however, the model that showed survival differing by sex and *p* differing across time had equal support (indicated by a $\Delta AIC < 2$). Phi under the former model was 0.82 (95% CI = 0.50-0.95) and under the latter male φ = 0.86 (95% CI =0.40-0.98) and female φ = 0.76 (96% CI = 0.47-0.91). Capture probability ranged from 0.06-0.17, depending on year.

Population size (N) estimated by POPAN5 was 681 (95% CI = 576-821) under the best model, which also supported φ differing between sexes and *p* varying by year. The estimates of φ by POPAN5 were 0.86 (95% CI = 0.477-0.98) for males and 0.65 (95% CI =0.43-0.83) for females. Estimates of *p* were congruent with those from the CJS analyses. Estimates of N were similar for the top several models. Probability of entry into the population (through birth or immigration) was 0.086 (95% CI = 0.04-0.16).

DISCUSSION

Our growth data represent the first such published data from a wild population of *T. g. gaigeae*. Expectedly, given their sexual dimorphism, adult females were found to grow more quickly than adult males. Carapace lengths of

both males and females were smaller than those reported for the species in New Mexico (Degenhardt et al. 1996).

Sex ratio can be an important factor in the dynamics of any population and has been deemed especially so in some turtle populations (Gibbons 1990). The sex ratio we report is more male biased than ratios from studies of New Mexico populations (1:1 and 1.48:1, Stuart 1998). While biased sex ratios in turtles may often be attributed to sampling bias there may also be underlying biological causes. In a recent study of *T. scripta*, it was concluded that females were more likely to escape from hoop nets than males (Brown et al. (in press)), although their probability of escaping was still very low and unlikely to cause as large a bias as we observed here. Indeed, biased sex ratios have been found in many wild turtle populations (Gibbons 1990, Lovich and Gibbons 1990, Janzen 1994, Gibbs and Steen 2005) with varying explanations.

Gibbons (1990) provides several explanatory mechanisms for biased sex ratios in turtles (other than gear selection). One is the possibility of bias being the result of differential production of male and female hatchlings in turtle species that undergo temperature dependant sex determination (TSD). *Trachemys g. gaigeae* are expected to be type 1A TSD like their congeners (Ernst and Lovich 2009), though this has not been confirmed. However, if this were the case, lower incubation temperatures would lead to higher production of males. It has been argued, however, that the long reproductive lifespan of female turtles would mediate this effect over time (Lovich and Gibbons 1990). It has also been

demonstrated that vegetative cover can influence the sex of hatchlings (Janzen 1994). This makes the case of *T. g. gaigeae* interesting because exotic vegetation has been colonizing the riverbanks in the Big Bend region for many years, to such a large extent that stream channel morphologies have been affected. This may have limited the availability of nesting habitat, or at least increased the proportion of it that was cooled by vegetation, potentially contributing to greater production of male hatchlings. It also could have increased the distance necessary for females to travel in search of nesting habitat, possibly exposing them to a greater risk of predation. Another mechanism proposed by Gibbons (1990) was differential mortality of the sexes. This has often been mentioned in the literature in relation to greater road mortality of females during nesting forays (Aresco 2005, Gibbs and Steen 2005). The necessity of nesting obviously also exposes females to increased risk of predation. While road densities in the Big Bend region have not increased proportionally to more populated regions of the United States, in some areas roads and parking areas are proximal to the river, and a few road mortalities of turtles have been observed. While this is unlikely to be significant enough to cause a large change in the sex ratio by itself, the scarcity of resources in the desert environment as well as the increased habitat for predatory mammals created by the exotic vegetation make it likely that predation risk is high for aquatic turtles during terrestrial forays. We found several carcasses left by predators (mostly raccoons) during this study but discovery of such remains is

generally very unlikely due to the impenetrable nature of the vegetation along the river. The fact that models showing lower apparent survival of females received strong support in our analyses lends additional support to the contribution of differential mortality to observed sex ratios in this case. On several occasions gill nets were observed set in the river by local Mexican fisherman. Aside from potential for mortality from being submerged in nets, it is likely that if significant harvest of turtles for food occurs, the larger females would be preferred. Females are also inherently more exposed to predation or collection by humans during the nesting season and may have to search a great distance to find nesting habitat in this harsh environment. There may be other sources of differential mortality in the population as well. The last mechanism proposed by Gibbons is differential maturation rate, where the more rapidly maturing sex will be more abundant in the population. Male biased sex ratios in red-eared sliders (*Trachemys scripta elegans*), a close relative of *T. g. gaigeae* (Jackson et al. 2008), and diamondback terrapins (*Malaclemys terrapin*) have been attributed to this feature found in some turtle species (Gibbons 1990, Lovich and Gibbons 1990). Indeed, this probably contributes to the sex ratio observed in this study as well, but not to the exclusion of other factors.

Model selection procedures identified two CJS models with essentially equal support. Both assumed that *p* varied across years, but the models supported different estimates of ϕ. The supported models (models 1 & 2) suggested that survival was constant throughout the study and but they differed

in that model 1 supported no difference in ϕ between the sexes whereas model 2 did. The fact that the best model selected in the POPAN5 analysis also supported a difference in ϕ between the sexes and the congruence of the ϕ estimates (and their confidence intervals) between the two analyses supports the conclusion that ϕ differs between male and female *T. g. gaigeae*. This is not unexpected given the additional exposure to predation and other risks that females must face during the nesting season. It is likely that there may also be a size selection effect on female survivorship, as observed in a study of predation on nesting female *T. scripta* (Tucker et al. 1999).

The population size estimated is small, but very well may be reflective of the heavy impacts upon this population and the ecosystem in general. Given the relatively few recaptures, it is possible that the population size was underestimated, however, the relatively narrow confidence interval indicated that our estimate likely reflects an accurate snapshot of N during our study period. This is a small population given the length of river sampled, and suggests that further protection and monitoring of this species is warranted. These results also suggest that there may have been a reduction in the suitable habitat in this region. While there were some instances of extreme flooding over the study period, there were also times when the river channel was dry or nearly so in some areas. Low water periods clearly made some large areas unsuitable and apparently uninhabited by turtles, at least for some period of time. This may also contribute to the skewed sex ratios if larger females are less able to cope with or

migrate from areas if they become unsuitable habitat. The fact that most recaptures occurred at the original capture site suggests that site fidelity is high in this population. This may seem to be contradicted by the low recapture rate, however this would not be contradictory if mortality (or other permanent removal) was higher than generally expected in the population. While turtles are capable of long life spans under ideal conditions, it is difficult using these methods to determine deaths in the population as recovery of dead individuals ranges from unlikely to impossible and cannot be parsed from emigration. The moderate apparent survival rate estimated in this study may be indicative of relatively high predation risk (human or otherwise) in this environment. During a radio telemetry study of *T. g. gaigeae* conducted in New Mexico, mortality was higher than expected at the end of the two year study, some of which were obvious results of predation (S. D. Moore and J. Jackson, unpublished data). The movements of individuals in our Big Bend study demonstrate that while shorter movements are much more common, this species has great dispersal capability in this environment.

In conclusion, *T. g. gaigeae* in the Big Bend region warrant continued concern given moderate adult survivorship and low abundance. This study provides new information about a wild population of this species, and background data for successful monitoring of this species in the future.

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Table 1. Mark-Recapture Model Selection Results. Candidate models and model selection results used for estimating ϕ and *p* of 270 adult male and 175 adult female *Trachemys g. gaigeae* captured in the Big Bend region of Texas from 2005 to 2010. Models are listed from most to least supported based on AIC_c scores. *t* represents time specific estimates (one estimate available for each year), **.** indicates estimates were constant across years and *sex* represents different estimates for each gender.

Rank	Model	AIC_c	ΔAIC_c	AIC_c weight
1	$\phi_{.}\ p_{t}$	588.738	0	0.40311
2	$\phi_{sex}\ p_{t}$	588.834	0.0967	0.38409
3	$\phi_{t}\ p_{t}$	591.733	2.9953	0.09016
4	$\phi_{t}\ p_{.}$	593.476	4.7388	0.03771
5	$\phi_{t}\ p_{sex}$	594.143	5.4057	0.02701
6	$\phi_{.}\ p_{sex*t}$	594.802	6.0648	0.01943
7	$\phi_{sex}\ p_{sex*t}$	594.866	6.1282	0.01882
8	$\phi_{sex*t}\ p_{t}$	597.23	8.4922	0.00577
9	$\phi_{.}\ p_{.}$	598.321	9.5829	0.00335
10	$\phi_{sex*t}\ p_{.}$	598.581	9.8429	0.00294
11	$\phi_{sex}\ p_{.}$	599.081	10.343	0.00229
12	$\phi_{.}\ p_{sex}$	599.725	10.987	0.0016
13	$\phi_{t}\ p_{sex*t}$	599.856	11.118	0.00155
14	$\phi_{sex*t}\ p_{sex}$	600.529	11.792	0.00111
15	$\phi_{sex}\ p_{sex}$	601.028	12.29	0.00086
16	$\phi_{sex*t}\ p_{sex*t}$	604.662	15.924	0.00014

Table 2. Average Measurements. Average carapace length (CL), carapace width (CW), plastron length (PL), plastron width (PW), body depth (BD) and mass of 270 male and 175 female *Trachemys g. gaigeae* captured over the course of the 5-year study in the Big Bend region of Texas. Standard deviations of the mean are in parentheses.

	CL	**CW**	**PL**	**PW**	**BD**	**Mass**
Males	135.27	102.17	120	79.77	46.2	328.3
	(19.07)	(12.21)	(16.34)	(11.34)	(6.95)	(133.24)
Females	178.84	134.36	165	107.58	67.25	836
	(28.73)	(19.03)	(26.3)	(15.07)	(12.44)	(330.63)

Table 3. Mean Annual Growth of Adult *T. g. gaigeae* in Texas. Mean annual growth in carapace length (CL) and mass of *Trachemys g. gaigeae* recaptures over the 5-year study period in the Big Bend region of Texas. Standard deviations are in parentheses.

	CL	Mass
Males (n = 45)	1.77 (2.53)	7.73 (49.43)
Females (n = 25)	3.14 (7.27)	23.63 (100.45)

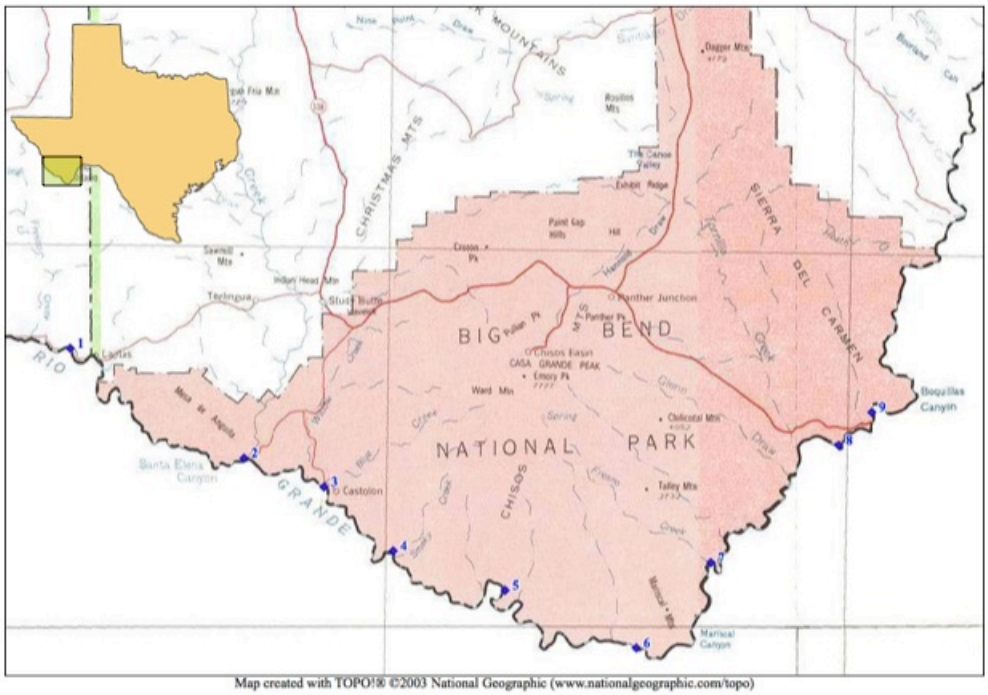

Figure 1. Big Bend sampling locations. Approximate locations of the nine sites sampled (blue circles) during the 5-year *Trachemys g. gaigeae* capture-recapture study in the Big Bend region of Texas.

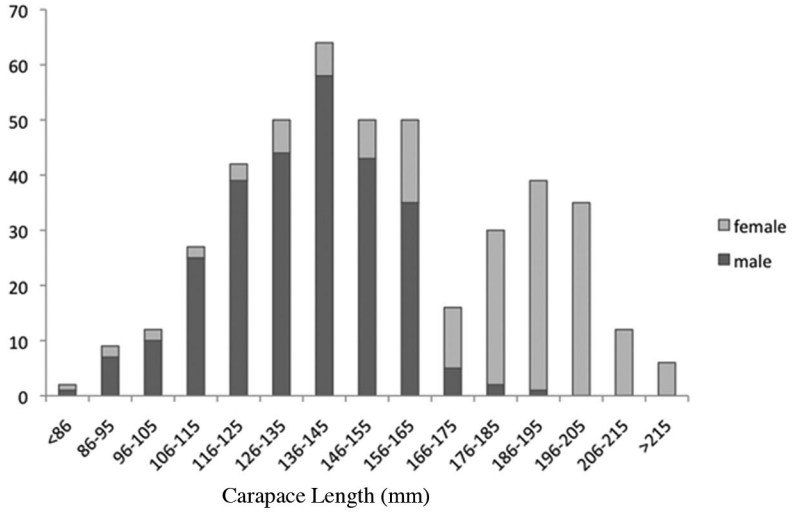

Figure 2. Length frequency histogram of *T. g. gaigeae* captures. Length frequency histogram of male and female *Trachemys g. gaigeae* captured during the 5-year study in the Big Bend region of Texas.

CHAPTER VI

SUMMARY

Analysis of molecular data in this study supports the species status of *T. gaigeae* as proposed by several authors (Seidel, 2002; Seidel et al., 1999; Ward, 1984; Weaver and Rose, 1967). My intention here, however, was to determine if this lineage is unique and worthy of treatment as a unit for conservation, rather than contribute to the overabundance of literature arguing the appropriate criteria for "species" definition. In conclusion, it appears that when mtDNA data are considered, the taxonomy of *Trachemys* proposed by Seidel (2002) is the most reasonable for the genus.

Growth data from analysis of mark-recapture from the Big Bend population represent the first such published data from a wild population of *T. g. gaigeae*. Expectedly, given their sexual dimorphism, adult females were found to grow more quickly than adult males. Carapace lengths of both males and females were smaller than those reported for the species in New Mexico (Degenhardt et al., 1996). The sex ratio reported here is more male biased than ratios from studies of New Mexico populations (Stuart, 1998). While biased sex ratios in turtles may often be attributed to sampling bias there may also be underlying biological causes. *Trachemys g. gaigeae* in the Big Bend region warrant

continued concern given moderate adult survivorship and low abundance. This study provides new information about a wild population of this species, and the necessary data for successful monitoring of this species in the future.

The occurrence of hybridization as observed in this study is a real threat to the genetic integrity of the Rio Grande endemic *T. g. gaigeae*. This alone justifies extensive *T. s. elegans* removal efforts within the historical range of *T. g. gaigeae*. Educational and enforcement efforts to discourage the release of captive *T. s. elegans* should also be implemented. The results of this study with regard to the identity of individuals from the Langtry area suggest that there is some potential for a hybrid zone to exist in this area, consequently, further study is warranted to elucidate the relationship between native Rio Grande *T. s. elegans* and *T. g. gaigeae* distribution in the main stem of the Rio Grande above Amistad Reservoir.

Currently, *T. g. gaigeae* populations receive little effective protection (Stuart and Ward, 2009). Given the population structure, low genetic diversity, low effective population size and directly estimated population size determined in this study, the conservation status of *T. g. gaigieae* should be elevated to endangered or even critically endangered. Determining between these two extreme levels of vulnerability requires assessment of the Mexican populations of this taxon. This is especially relevant due to the introduction of turtles from other populations that are now documented to occur in both NM and TX and subsequent threats from hybridization, as well as the continuing and increasing

demands on the river that provides them with habitat (Hoyt, 2002; Wong et al., 2007).

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VITA

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