THE STATUS OF *PSEUDEMYS GORZUGI* (THE RIO GRANDE RIVER COOTER) IN TEXAS RIVERS

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ABSTRACT

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Many turtle species are threatened by changes to their habitats, introduced species, and exploitation for human uses including food, medicines, and pets. *Pseudemys gorzugi*, the Rio Grande River cooter, is listed as endangered in New Mexico. In order to determine if similar conservation measures are warranted in Texas and to establish successful management strategies, it is essential to have a basic understanding of the life history and population genetics parameters of this species. The goal of this research was to construct a profile of *Pseudemys gorzugi* that described the evolutionary and life history of the species including details about the population density, ecology, genetic structure, and any outside factors that may be negatively impacting the population. Extensive surveys of both the historical and current distribution of this species in Texas revealed a low population density and a paucity of juveniles in the population. An

examination of basking habits revealed a late afternoon peak in basking activity of the *Pseudemys gorzugi* population monitored during the summer of 2004. Dietary fecal analysis showed that the majority of the diet was composed of macrophytic algae. Based on genetic analysis of the mitochondrial ND4 gene, *Pseudemys gorzugi* appears to be monophyletic within a limited taxonomic sampling of emydids. Genetic analyses of both the mitochondrial ND4 gene and five microsatellite DNA markers indicated the population is homogeneous throughout its range. In addition, research revealed evidence of multiple threats common to extinction events in chelonian populations, including habitat degradation, the introduction of fire ants, and over-collection for the pet trade. This research provides state management authorities with essential data to determine if additional conservation efforts are necessary to protect this unique west Texas species.

INTRODUCTION

In 1990, the New Mexico Department of Game and Fish listed *Pseudemys gorzugi*, the Rio Grande River cooter, as an endangered species (Degenhardt et al., 1996). In order to determine if similar conservation measures are warranted in Texas and to establish successful management strategies, it is essential to have a basic understanding of the natural history and population genetic parameters of this species (Schwartz et al., 2003). The goal of this research is to construct a profile of *Pseudemys gorzugi* that describes the evolutionary and life history of the species including details about the population density, ecology, genetic structure, and any anthropogenic factors that may be impacting the population. The following account provides a detailed description of *Pseudemys gorzugi* as it occurs in Texas and provides state government officials with the necessary data to determine if additional conservation efforts are required to protect this unique species.

Turtle Biology

Emydidae.— The emydids are predominately New World turtles with the exception of the European-Southwest Asian genus *Emys* (Zug, 1993). The moderately sized turtles in this family represent the most abundant, speciose and ecologically diverse family of turtles in North America (Ernst et al., 1994). Currently, there are 40 recognized

species placed within ten genera. The group has been studied extensively since the mid 1800's and remains one of the most popular research units in field biology (Gibbons, 1990). Despite the amount of research examining emydids, many questions remain concerning evolutionary relationships, taxonomic status, and the population biology of these animals.

There are two major subfamilies: the Emydinae (*Clemmys, Emydoidae, Emys, and Terrapene*) and the Deirochelyinae (*Chrysemys, Deirochelys, Graptemys, Malaclemys, Pseudemys, and Trachemys*) (Stephens and Wiens, 2003). Emydidae contains carnivores and herbivores in both aquatic and terrestrial systems. Within the family, the basal split among the genera appears to correspond to a shift between aquatic and semi-terrestrial habitat use; more recent speciation events occurred among geographically distant taxa (Stephens and Weins, 2003). These speciation events can be attributed to allopatric speciation within ecologically similar taxa (Stephens and Weins, 2003). While early diversification in the family seems to have been driven by competition, with time, non-ecological mechanisms like allopatric speciation and sexual selection, will drive speciation (Stephens and Weins, 2003).

Pseudemys.— The turtles in this genus are some of the most conspicuous and abundant basking turtles in the ponds and streams of the eastern United States and northeast New Mexico (Iverson, 1992). In general, river cooters are moderately sized turtles reaching carapace lengths up to 43.7 cm (Pritchard, 1980).

Three species of *Pseudemys* occur within Texas (Dixon, 2000). *Pseudemys concinna metteri*, the Missouri River cooter, is somewhat smaller than other representatives in the genus. They can be distinguished by a lack of a post-orbital spot

and lack of concentric whorls in the second costal scute, which may or may not contain a light C; there is also a pattern of dark lines on the seams of the plastron (Conant and Collins, 1998). *Pseudemys concinna metteri* can be found from south-central Missouri and adjacent southeast Kansas, south through eastern Oklahoma, western Arkansas, extreme northwest Louisiana, and eastern Texas through the Gulf of Mexico (Conant and Collins, 1998).

Pseudemys texana can be distinguished by yellow head markings with lateral stripes, a vertical bar of yellow at the angle of the jaw, and a small, round post-orbital spot. The second costal scute contains five or six concentric whorls with dark centers, and the plastron has dark lines along the seams. The upper jaw has a central notch bordered on either side by a cusp; a pair of swollen ridges extends downwards from the nostrils to terminate the cusps. *Pseudemys texana* can be found throughout most of central Texas, from San Antonio Bay and Galveston on the Gulf of Mexico, extending west into the Colorado, Brazos, Guadalupe, and San Antonio river drainages (Conant and Collins, 1998).

The other Texas *Pseudemys* is one of the least known species in the genus; it is also one of the least known Texas turtle species. The Rio Grande River cooter, *Pseudemys gorzugi*, is usually characterized as a locally abundant but uncommon turtle in three Texas river systems. The taxon extends northward into New Mexico where it is a state protected wildlife species (Degenhardt et al., 1996).

Pseudemys gorzugi.— *Pseudemys gorzugi* (Ward, 1984), the Rio Grande River cooter, had been formerly considered a subspecies of *P. concinna* (Le Conte) (Ernst, 1990; Collins, 1991). Ernst (1990) elevated the individuals found in the Rio Grande and

Pecos river drainages to full species status and justified his position based on the lack of genetic exchange between these specimens and other *Pseudemys concinna* populations. The distinction was also supported by the examination of morphological characters; the turtle does not have the usual "C" markings on the plastron, but instead black and yellow concentric circles (Degenhardt et al., 1996).

Pseudemys gorzugi is a large freshwater turtle with an elongate oval carapace that is highest at the middle and widest behind the midline. The carapace has an intricate pattern of green, yellow and black markings; the second costal scute has a unique concentric circle in place of the C-shaped mark found in other species of the genus in Texas. Older males may become melanistic obscuring much of the carapace design with vermiculations of black on a reddish or gray background (Figure 1). This pattern in adult male *P. gorzugi* may well represent a unique feature of the species, as we are unaware of this age-dependent patterning in other species of *Pseudemys* (Bailey and Forstner, 2004, in press). Such patterning, however, has been reported in *Chrysemys* (Ultsch, 1999).

Hatchlings have a central keel that disappears with age. Their skin is olive to brown, with characteristic yellow stripes on the head and the neck and large blotches on the side of the head. The surfaces of the upper and lower jaws have well-developed denticulations; the upper jaw also has a medial notch bordered by tooth-like cusps (Degenhardt et al., 1996).

Sexual dimorphism is pronounced in most populations; females tend to grow to greater lengths and greater carapace heights than do males. Males have a broader tail and may have a slightly concave plastron. The anal opening in males extends past the distal edge of the carapacial margin and males also have long, straight fore claws that are used

in courtship displays (Degenhardt et al., 1996). In those emydid genera known to utilize foreclaw titillation as an elaborate aquatic courtship display, the male faces the female, head-to-head, vibrating his foreclaws near or against the female's eyes (Seidel and Fritz, 1997). *Pseudemys* is the only one of these genera where the male approaches the female, and while facing the same direction, vibrates his foreclaws toward the female from above (Seidel and Fritz, 1997). The trait is used as a synapomorphy for the genus since this behavior has not been observed in any other Deirochelyine turtles (Seidel and Fritz, 1997).

Pseudemys gorzugi generally occurs in riverine habitats, primarily in deep pools of high flow areas. Individuals may be found in muddy, sandy, or rocky areas, or in areas of algae-covered limestone that appear to serve as suitable habitat for the species. Aquatic vegetation is preferred for foraging and protection, but the lack of macrophytic vegetation will not exclude the species from an area. The average elevation for the habitat is around 1000m in New Mexico (Degenhardt et al., 1996), but elevation data has not been reported for Texas.

The Rio Grande River cooter is a unique component of clear water spring and river systems in the South Texas Plains and Edwards Plateau regions of Texas (Devils, Pecos, and Rio Grande river systems). It is found in the lower Rio Grande and Pecos river drainage basins from Tamaulipas, Nuevo Leon, and Coahuila, Mexico and south Texas, through west Texas, to southeast New Mexico. *Pseudemys gorzugi* is found in a narrow band following the Rio Grande south to Del Rio, and then branching off into the Devils and Pecos rivers. There are also disjunct populations in the Guadalupe Mountains (Degenhardt et al., 1996) of New Mexico.

Anthropogenic Changes to Texas Rivers

Modification to the flow rates of Texas rivers has drastic impacts on the environment and consequences for the organisms found in these drainage systems. The construction of dams, flood-control practices, channelization, water diversions and the introduction of tamarisk (salt cedar) have caused the Rio Grande River to become increasingly intermittent (USDOI, 1998). These practices have significantly degraded the water quality and often result in little to no surface water flow on the river (USDOI, 1998). The reduction to in-stream flow rates negatively impacts the environment since there is no longer enough water to support the aquatic and riparian habitats while simultaneously meeting the current levels of human consumption (USDOI, 1998).

The Rio Grande River in west Texas has three major tributaries: the Rio Conchos (26,404 m^2 watershed), the Pecos River (35,308 m^2 watershed), and the Devils River (4,305 m^2 watershed) (USDOI, 1998). The Rio Grande River rises in Colorado, flows southward through New Mexico, forming the international boundary between Mexico and Texas. The river is approximately 1,896 miles in length; approximately 1,248 miles form the southern boarder of Texas. The Pecos River rises in Mora County, New Mexico flowing southeast into Texas to join the Rio Grande River. The river is approximately 500 miles in length. Much of the river is narrow, water levels are generally low and the flow rate is minimal. As the river continues southward through the arid climate of the Chihuahuan Desert, an extensive network of canyons has been carved out. The Devils River rises in Sutton County, Texas, flowing south through Val Verde County where it joins the Rio Grande River just outside of Del Rio. The river is approximately 100 miles in length but due to the arid nature of the region, it is intermittent through much of this

area. The Devils River is spring-fed throughout the 100-mile stretch and many of the creeks that flow into the Devils River are also spring-fed. The river bottom is composed of sand and gravel, allowing for high levels of visibility (http://www.tpwd.state.tx.us/conserve.htm).

Before 1915, the lower Rio Grande flow was virtually unimpeded. The Rio Grande was impounded at Amistad Dam in 1969; the Pecos and Devils rivers contribute flow directly into the reservoir at Amistad Dam. The Devils River mean annual flow is twice that of Pecos despite their discrepancy in size. The seemingly contradictory higher flow rate occurs because the Devils River is in mostly semi-arid environment as opposed to the arid environment of the Pecos; unlike the Devils, there are high amounts of alluvial deposits in the Pecos, and there are significantly higher numbers of water diversions for irrigation (USDOI, 1998).

Surveys indicate that *Pseudemys gorzugi* frequently inhabit deep, clear-water river pools with abundant macrophyte vegetation availability. Human changes to the environment have drastically decreased the flow rate for nearly all rivers in which *P. gorzugi* are found. In addition, pollutants are also likely to be impacting the habitat quality of these rivers. Available data from recent studies by the USGS (http://water.usgs.gov/nasqan/data/statsum/pecos.html) determined significant levels of atrazine, a known endocrine disruptor, in the Pecos River system. Untreated sewage from Mexican border villages, runoff from agriculture and mining activities, and atmospheric deposits are some of the point and nonpoint sources that contribute to the declining water quality in the Rio Grande River drainage system (USDOI, 1998). The Texas Natural Resources Conservation Commission has found arsenic, cadmium,

chromium, copper, lead, mercury, phosphorus, selenium, silver, zinc, DDD, DDE, DDT, dieldrin, endrin, hexachlorobenzene, PCB's and total PHA's at levels which are of concern to humans using this water system (Texas Water Commission, 1992a, 1992b; Texas Natural Resources Conservation Commission, 1994a, 1994b). The declining water quality of the Rio Grande has been shown to cause decrease in fish density and diversity, but few studies have examined the impact on other vertebrates (Bestgen and Platania, 1988).

Objectives

Human changes to the landscape of Texas are dramatic and are continuously increasing. Among those are impacts to groundwater resources and in-stream flow rates. Relatively little work in Texas has been published applying turtles as a biological monitor of the wildlife health of our streams and rivers. Despite considerable evidence from other states (USFWS, 1986, 1987) and our own (*Graptemys caglei*) that turtles provide remarkable value as biological indicators (Gibbons, 1990). In-stream water reductions coupled with increased harvest pressure on turtle species (IUCN/SSC, 1991) may be placing many Texas turtle species at considerable risk. The goal of this research is to provide information on the current status of the *Pseudemys gorzugi* population in Texas in order to evaluate trend data where possible, and to provide information that will be specifically useful in determining appropriate non-game harvest rates and warning signs of population decline or extirpation. Preliminary work indicated troubling declines and a lack of recruitment, thus the research sought to determine if there is indeed a downward trend in the abundance of Rio Grande River cooters in Texas, and if so, to evaluate

underlying causes and to seek methods to ensure self-sustaining populations of the species within the state.

MATERIALS AND METHODS

Field Work

Population Surveys.— Field surveys were conducted throughout the range of *Pseudemys gorzugi* in the Rio Grande River drainage system in Texas to assess the distribution and abundance of the species in the state. Since *P. gorzugi* is often collected for the pet trade (Appendix 1, Appendix 2), the specific locations of our sample sites have been intentionally removed from this account; upon request by qualified individuals this information may be available from the author.

Specimens were collected from fourteen specific locations (Figure 2, Table 1). Individuals were collected using seining, hoop or basking log traps, as well as physical collection using a snorkel or SCUBA apparatus (Anderson, 1965). Once the turtles were captured, a small aliquot of blood was drawn (approximately 1ml) from the femoral vein and then placed into blood storage buffer (100mM Tris: 100mM EDTA: 2% SDS) while in the field. Additionally, the turtles were marked using a system of notching the marginal scutes (Cagle, 1939) so that recaptured individuals could be identified. The specimens were measured (carapace length, carapace width, plastron length, plastron width, and body depth; cm), weighed (g), and the data was recorded. The morphological data was compared to similar data for *Pseudemys gorzugi* from New Mexico (Degenhardt et al., 1996) and *Pseudemys texana* samples from the San Marcos River to determine if

differences existed between the locally abundant *P. texana* individuals and the more rare *P. gorzugi* individuals.

Data were also collected for 14 *Pseudemys gorzugi* hatchlings born in captivity. The specimens were measured (carapace length, carapace width, plastron length, plastron width, and body depth; cm), weighed (g), and the data was recorded. The data were used to compare the sizes of *P. gorzugi* and *P. texana* hatchlings to determine if differences existed between the locally abundant *P. texana* individuals and the more rare *P. gorzugi* individuals.

Data were recorded in an attempt to determine the abundance of *Pseudemys gorzugi* in the Pecos and Devils Rivers. A 60-mile stretch of the Pecos River and a 22-mile stretch of the Devils River were surveyed and the numbers of turtles observed in each species present were recorded. For comparison, the number of *Pseudemys texana* per river mile was recorded for the San Marcos River using the same methods. A 5.5-mile stretch of the San Marcos River was surveyed in late May and late September in each of two years. It should be noted that the data collected in late September of the first year were collected directly following a large flood. The number of specimens collected per river mile was recorded for use in comparison with similar data previously collected for *P. gorzugi.*

Thermal Ecology.— Basking is characteristic of emydid turtles, and this behavior has been extensively investigated in members of this family (Cagle, 1950; Boyer, 1965; Moll and Legler, 1971). There are two predominant forms of basking observed in turtles; aquatic basking, where the turtle floats at the surface of the water, and atmospheric basking, where the turtle is out of the water and exposed to full sunlight (Moll and Leger,

1971). Baking functions in thermoregulation, in the drying of the shell, and in the removal of algae and ectoparasites from the shell (Boyer, 1965). Turtles will often alter their body position and extend their appendages to increase the amount of surface area exposed to the sun (Crawford et al., 1983). The basking habits of *Pseudemys gorzugi* were investigated to determine the peak hours of activity for the species in Texas.

The Thermocron iButton, manufactured by Dallas Semiconductors (Dallas, Texas, USA), was used to record temperature at user specified intervals. These environmental sampling thermocrons are currently being employed in behavioral and physiological ecology as an inexpensive and accurate alternative to other systems (Angilletta and Krochmal, 2003). Each thermocron is 5.9 mm thick with a diameter of 17.4 mm and weight of 3.1 g. The thermocrons has 512 bytes of memory and uses iButton reader (Blue Dot Receptor, Model DS1402D-DR8) in conjunction with software (32-Bit iButton-TMEX Runtime Environment) to display the temperature records.

Within one of the study populations, 20 *Pseudemys gorzugi* were tagged with thermocrons. They were attached to the rear carapace of the individuals using marine epoxy; the data-loggers were positioned so as not to interfere with copulation. The thermocrons were deployed in three subsets, where the first group of loggers was set to record temperatures every hour, the second group every two hours, and the last group every four hours. After six weeks, the individuals tagged with the thermocrons were recaptured, when possible, and the thermocrons removed in order to retrieve the data. During the study period a Thermocron iButton was also deployed in the river itself to record the environmental fluctuations in the river at a depth of 1m.

Laboratory Work

Dietary Fecal Analysis.— Pseudemys are primarily vegetarian, consuming the natural aquatic vegetation found in the area, although many of these turtles will take raw meat, fishes, shellfishes, worms and insects (Conant and Collins, 1998). It has been shown in *Pseudemys concinna*, that symbiotic bacteria in the gut of the turtle aids in digestion (Thomas et al., 1994).

Fecal samples were collected from a subset of the *Pseudemys gorzugi* specimens sampled. The fecal sample was preserved in 95% ethanol. The feces were examined under a dissecting microscope, separated into categories, and the volumes were quantified using water displacement (Dreslik, 1999). The samples were separated into five categories: 1) arthropods, 2) crustaceans, 3) algae, 4) macrophytic algae, and 5) macrophytic vascular plants. The percent volumes were calculated for each of the five food types and the percent relative volume was calculated for each individual (Dreslik, 1999).

Mitochondrial DNA Analysis.— Mitochondrial DNA has been used to analyze natural populations since the late 1970's (Avise et al., 1979). Mitochondrial DNA is useful in these studies because it is maternally inherited, haploid, and has a high mutation rate. The mitochondrial ND4 gene was sequenced to determine the amount of variation among *Pseudemys gorzugi* from the sample locations as well as to compare the sequence with other *Pseudemys* found in Texas. Genomic DNA was extracted from blood samples using the Qiagen DNeasy Tissue Extraction Kit in accordance with the manufacturers specifications. The samples were then amplified via the polymerase chain reaction (PCR) using the primers ND4 (5'—3': CAC CTA TGA CTA CCA AAA GCT CAT

GTA GAA GC) and LEU2 (5' – 3': TGC TTT TAC TTG GAT TTG CAC CAA G). The hot start PCR method was used with the following thermal cycling parameters: 1 cycle of 95° C for 5 minutes, then 40° cycles at 95° C for 30 seconds, 50° C for 1 minute, 72° C for 1 minute, followed by one cycle of 72° C for 5 minutes. The PCR products were purified using the Promega Wizard SV Gel and PCR Clean-up System in accordance with the manufacturers specifications. The purified PCR products were cycle sequenced using the following thermal cycling parameters: 25 cycles at 96° C for 10 seconds, 50° C for 5 seconds, and 60° C for 1 minute. The cycle sequence products were purified using Princeton Separations Centri-Sep Columns in accordance with the manufacturers specifications. The purified products were then directly scored using the ABI 377XL PRISM Automated DNA Sequencer.

Analyses using both parsimony and distance based methods were used to construct a phylogenetic hypothesis for the mitochondrial sequences (Hillis et al., 1996). The mitochondrial DNA sequences were aligned using Sequencher 4.2. The dataset was analyzed using PAUP* 4.0b10 (Swofford, 2003). *Trachemys scripta elegans* was set as the outgroup and the tree was rooted so that the outgroup and ingroup were monophyletic. The optimality criterion was set to parsimony and a heuristic search was conducted using TBR branch swapping. Starting trees were obtained using stepwise addition from a random addition sequence; 1000 replicates were performed and one tree was held at each step. A strict consensus tree of the two most parsimonious trees was produced. The CI and RI were recorded. The bootstrap is a statistical technique for estimating the amount of ambiguity in a proposed phylogeny and is a commonly used confidence estimator (Alfaro et al., 2003) or as a measure of repeatability (Hillis and

Bull, 1993). A bootstrap analysis was performed using 100 replicates and the percentage of support for each branch was recorded.

The distance optimality criterion allows for the correction of nucleotide substitutions within DNA sequence data and limits homoplasy (Nei and Kumar, 2000). Again using PAUP* 4.0b10 (Swofford, 2003) and the same outgroup, the optimality criterion was set to distance and the Neighbor-joining algorithm was employed to produce a phylogram base upon Jukes-Cantor corrected distances. The Jukes-Cantor model of nucleotide substitution assumes that nucleotide substitution occurs at any nucleotide site with equal frequency and that at each site a nucleotide changes to one of the three remaining nucleotides (Nei and Kumar, 2000). A bootstrap analysis was performed using 100 replicates and the percentage of support for each branch was recorded.

A spatial analysis of molecular variance (SAMOVA) was conducted to examine the partitioning of molecular variation in the population using the program SAMOVA (Dupanloup et al., 2002). SAMOVA allows for the partitioning of molecular datasets based on genetic homogeneity and geographic proximity without incorporating any *a priori* information (Dupanloup et al., 2002). Analyses were conducted with the number of groups (k) set from 1 to 11 and the data were recorded.

Microsatellite DNA Analysis.— Microsatellites are repetitive sequences of DNA distributed throughout the nuclear genome. They are presumably neutral (Tautz et al., 1986) and display higher mutation rates than observed in nuclear coding regions of the genome (Jeffreys et al., 1994). Microsatellites are ideal for studies examining the genetic diversity present in populations and allow for the examination of genetic relationships

between populations of a species. They are biparentally inherited, abundant in the genome, generally display high levels of polymorphism, and they are codominant markers. Species-specific microsatellite loci are preferred for population structure assessments (Aggarwal et al., 2004) since heterozygosity and polymorphism tend to decrease with increasing phylogenetic distance from the taxon for which the makers were developed (Ellegre et al., 1995). The analysis of microsatellite allele frequencies allows for estimates of gene flow, population sizes, and overall population heterogeneity (Smith and Wayne, 1996). Recent advances in the application of such data to derivation of population structure and spatial geography (Bowen and Avise, 1995; Bowen et al., 1994) can now be used to examine the relationships among the *P. gorzugi* populations in Texas.

Microsatellite loci were amplified in order to estimate the amount of genetic diversity present in *P. gorzugi* throughout the sample locations. The nuclear microsatellites available were developed for similar projects in the Big Bend slider (*Trachemys gaigeae*) (Forstner et al., 1999; Forstner et al., in review). These markers have proven polymorphic in all emydid genera. Five primer pairs were used to investigate the population genetics of *Pseudemys gorzugi* (Appendix 3). The primer combinations were optimized using PCR with the following thermal cycling parameters: 1 cycle of 95° C for 5 minutes, then 35 cycles at 95° C for 30 seconds, 50-60° C for 1 minute, 72° C for 1 minute, followed by one cycle of 72° C for 5 minutes. Once the optimal annealing temperature was determined, an additional PCR was performed using the afore mentioned thermal cycling parameters, along with fluorescently labeled forward primers and unlabeled reverse primers. The PCR products were then directly scored

using the ABI 377 Automated DNA Sequencer; the allele sizes were determined using GeneScan 2.1 (ABI, inc.).

The genetic diversity present within each *a priori* population was calculated using *Arlequin* version 2.000 (Schneider et al., 2000) and recorded as the number of alleles per locus (A), the observed heterozygosity (H_O) and the expected heterozygosity (H_E). Allele frequencies were calculated for each population at each of the five loci. An analysis of molecular variance (AMOVA) was conducted to test the hypothesis that the partitioning of genetic variation among populations occurred between the northern and southern study sites. For comparison, STRUCTURE version 2 (Pritchard et al., 2000) employs a Bayesian approach to partition the dataset into the most likely clusters based on allelic composition. Analyses were conducted with a "burn in", or parameter stabilization period, of 50,000 and a markov chain of 500,000 using a model allowing for admixture. The estimated ln probability of the data was recorded and subsequently applied in determining the clustering solution with the highest probability.

RESULTS

Field Work

Population Surveys.— In total, approximately 327 *Pseudemys gorzugi* specimens were observed and 189 (57% of the total) were collected, sampled, and measured (Figure 2, Table 1). An additional group of approximately 9 dead, complete or partial skeletal remains were located across the sampling sites during 2003-2004. During the surveys a new county record for *Pseudemys gorzugi* was found in Maverick county Texas (Bailey et al., 2004). In addition, the largest recorded individual *Pseudemys gorzugi* was collected from Val Verde county Texas (Stout et al., in press). During the surveys conducted from 2002-2004, fire ants were noted as far west as Langtey Texas. Fire ants were first observed by M.R.J. Forstner on the shore of Lake Amistad in 1998 and are now (2003) found in tremendous abundance (Forstner pers. comm.).

The population meristics of the recorded individuals, as well as comparative data for *Pseudemys gorzugi* populations in New Mexico (Degenhardt et al., 1996), and *Pseudemys texana*, are summarized in Table 2. A two-sample t-test (two-tailed version) was performed to compare the meristics of the two species. The test shows a statistically significant size difference between the two species for the carapace length, carapace width, and plastron length (Figure 3, Table 2). The ratio of *P. gorzugi* males to females to juveniles was 90:54:6 respectively. The adults ranged in size (carapace length) from 6.7-37.2 cm.

The population meristics for captive born *Pseudemys gorzugi* and *Pseudemys texana* hatchlings is summarized in Table 3. A two-sample t-test (two-tailed version) was performed and the results show a statistically significant difference in the carapace length between the two species even as juveniles (Table 3). The *Pseudemys gorzugi* hatchlings ranged in size (carapace length) from 33.6-42.4 mm.

The number of *Pseudemys gorzugi* observed per river mile was recorded for the Pecos and the Devils rivers (Table 4). A 60-mile stretch of the Pecos River was surveyed. Over the entire stretch, 123 adults were recorded (2.05 per river mile), and 3 juveniles were recorded (0.05 per river mile). A 22-mile stretch of the Devils River was surveyed. Over the entire stretch, 68 adults were recorded (3.09 per river mile), and 3 juveniles were recorded (0.14 per river mile). For comparison, the number of *Pseudemys texana* per river mile was recorded for the San Marcos River (Table 4). A 5.5-mile stretch of the river was surveyed in late May and late September in each of the two years of grant activity. On average, 85.8 adults were recorded over the entire stretch (15.6 adults per river mile); 18.7 juveniles were recorded over the entire stretch (3.4 juveniles per river mile). Obviously these numbers are drastically different from the numbers of *P. gorzugi*. It should be noted that the data collected on the San Marcos River in late September of the first year was done so directly following a large flood and is thus likely to be an underestimate of survey numbers resulting were it not for the flood event.

Thermal Ecology.— The Thermocron iButton data were collected from four specimens (Figure 4) as well as from the environmental sampling thermocron placed at a depth of 1M in the river. The temperature data retrieved was recorded at multiple time intervals depending on if the thermocron was originally set to record temperatures every

hour, every two hours, or every four hours. Because of these scale differences, daily temperature recordings at four-hour intervals were used for subsequent analyses. The daily temperature data were then averaged across the six-week period in which these measurements were taken. The temperature data from the turtle's thermocrons were then compared to the temperature data from the environmental sampling thermocron; the temperature peak occurs at hour 16, or 4 p.m. (Figure 4).

Laboratory Work

Dietary Fecal Analysis.— The fecal analysis showed that by volume 69.66 % of *Pseudemys gorzugi* diet was composed of filamentous green algae, 14.94% macrophytic vascular plants, 7.47% macrophytic algae, 6.97% arthropods, and 0.96% crustaceans (Table 5).

Mitochondrial DNA Analysis.— A total of 909 bases resulted from the sequencing of the ND4 gene region in the *Pseudemys gorzugi* samples tested. Analysis of the mitochondrial DNA sequence revealed one polymorphic site resolving two haplotypes; at position 360, there was a transistion between C and T. The nucleotide diversity value was 0.001. The parsimony analysis of the data in PAUP* 4.0b10 (Swofford, 2003) produced two equally parsimonious trees. High levels of bootstrap support resulted for the nodes resolved on the bootstrap tree (Figure 5). The CI was 0.988 and the RI was 0.975 revealing minimal amounts of homoplasy within the dataset. The phylogram from the Neighbor-joining analysis using the Jukes-Cantor distance correction method is shown in Figure 6. The topologies for the parsimony and distance trees were identical. The SAMOVA analysis revealed that with *k* set at two, 69.98% of

the genetic variation occurs between groups (Figure 7). At other values of *k*, less genetic variation is explained by the groupings.

Microsatellite DNA Analysis.— The genetic diversity present within each population was calculated for each locus using *Arlequin* (Schneider et al., 2000) and recorded as the number of alleles per locus (A), the observed heterozygosity (H_O) and the expected heterozygosity (H_E). All loci were polymorphic, showing 2-21 different alleles per locus (average 6.8), H_O values ranging from 0.143-1.00, and H_E values ranging from 0.33-1.00 (Tables 6-10). The allele frequency distribution shows the presence of some common alleles throughout the range as well as private alleles or different frequencies of alleles from that found in adjacent populations. The results of the AMOVA analysis indicate that only 13.8% of the genetic variation occurs between the northern and southern study site locations (Figure 8). STRUCTURE version 2 (Pritchard et al., 2000) was used to analyze the allele frequencies at each locus and to determine how the dataset should be partitioned. STRUCTURE analyses, with *k* set from one to fourteen, revealed that three clusters appear to best explain the variation seen in the dataset (Figure 9). When *k* was set to three, individuals were assigned to clusters without regard to geographic origin (Table 11).

DISCUSSION

The most important benefit of this research is in providing a current depiction of the distribution and characteristics of *Pseudemys gorzugi* in all of the Texas river systems in which it occurs. Multiple factors appear to be threatening the remaining Texas *P. gorzugi* populations. An overall low population density, the lack of evidence of significant recruitment, anthropogenic changes to the habitat, toxins and the novel presence of imported fire ants (*Solenopsis invicta*) represent factors that are actually or likely impacting *P. gorzugi* in Texas. It appears that very little genetic variation is present among the populations of *Pseudemys gorzugi* in Texas, which could limit the species ability to adapt to environmental changes.

Population Surveys

The historical range of *Pseudemys gorzugi* spans the length of the Rio Grande, Pecos and Devils river drainages; the occupied range of *P. gorzugi*, however, is much smaller than the available range (Figure 7). A comparatively very low population density of *Pseudemys gorzugi* relative to *Pseudemys texana* was detected for the rivers sampled within Texas (Table 4). The low population density, coupled with existing threats and direct anthropogenic mortality, poses a considerable threat to the persistence of this species in Texas. The results from both the long term studies (Appendix 2) and the data

collected over the last few years also show a conspicuous lack of juveniles throughout the range (Table 1). Juvenile turtles are notoriously difficult to locate in the wild, however the sampling efforts directed towards locating *Pseudemys gorzugi* were unsuccessful whereas the same sampling efforts were successfully employed to locate juvenile *Pseudemys texana* (Table 4).

While the cause for the small numbers of juveniles is not yet known, it is certain that the lack of juveniles, and consequent recruitment, could pose a serious threat to the stability of the population in the future. The observation of nearly an order of magnitude difference in the number of juveniles observed per river mile between *P. gorzugi* and *P. texana* supports this conclusion (Table 4). The values provided for the Pecos and Devils rivers are likely overestimates if extrapolated to the actual currently occupied *P. gorzugi* habitat in each of the rivers. Densities of the species in significantly compromised areas of the Pecos River, or within the Rio Grande River itself, are so low that collection of individuals in those areas was unsuccessful.

Thermal Ecology

The application of thermocron iButtons in the examination of thermal ecology in wild free-ranging *Pseudemys gorzugi* represents a new tool for turtle field biologists. While these devises have been used to study nest cavity temperatures (Angilletta and Krochmal, 2003), the use of these environmental temperature loggers to monitor free-ranging vertebrates has not been previously published. The application of this tool in the study of *P. gorzugi* represents a significant advance for the ecological examination of not just this species but of many turtle species. The thermocron results provide preliminary

data regarding the basking habits of *P. gorzugi* in west Texas. For the late spring and summer months logged, the average temperature of the turtles is always well above the mean temperature of the river and that temperature spikes in late afternoon when the highest probability of substrate basking occurs (Figure 2). The partitioning of different basking behaviors is important in this study, as we believe the results support our use of observational (sight) surveys in the determination of the density of turtles in these rivers. The environmental monitoring indicates that these turtles are actively basking during the day, allowing for a higher probability of visual encounters during our field surveys.

Research on basking behavior has yielded results similar to those seen in this study. A mid-afternoon peak in basking activity has also been observed in *Pseudemys concinna* populations in Illinois (Dreslik, 2000). Seasonal differences in basking behavior have also been documented in this genus (Auth, 1975), although not all *Pseudemys* species exhibiting these fluctuations (Moll and Legler, 1971).

Continued research using the thermocrons may provide additional evidence about the partitioning of float basking and substrate basking habits in *P. gorzugi*. Additional research is also underway to attempt to learn about the reproductive behaviors (i.e. when females are laying eggs and if they lay multiple clutches per year) using the same technology.

Dietary Fecal Analysis

The results of the dietary fecal analyses (Table 5) are similar to work done on *Pseudemys concinna* (Dreslik, 1999) which showed that 98% of their fecal matter was composed of two genera of filamentous algae. The two closely related turtle species

appear to be using the same foraging strategies. The diet of both species also seems to be correlated with the size of the turtles; the smaller turtles appear to be consuming a higher percentage of arthropods than the larger individuals. The differences could be due to differences in foraging strategies between juveniles and adults or due to the resource availability in the portion of the habitat occupied by juveniles and adults, since juveniles will stay in areas of denser vegetation to avoid predators.

In a study of the food habits of *Pseudemys texana* (Fields et al., 2003), the researchers found that 91.6% of the diet was comprised of aquatic macrophytes. The majority of those macrophytes were non-native, introduced plant species and found to have a lower nutrient content than the native macrophytic plants usually consumed by *P. texana* in the area; the data is useful in assessing the impact of non-native plants on aquatic fauna and can be used to develop models used in the determination of appropriate management strategies. As the number of non-native plant species in west Texas rivers increases, there could be consequences for *P. gorzugi* in terms of their subsistence strategies and the metabolic repercussions associated with differences in the nutrient quality of the plants in their diet.

Mitochondrial DNA Analysis

The phylogenetic analyses of the mitochondrial DNA dataset indicate that *Pseudemys gorzugi* represents a monophyletic group relative to *Pseudemys* species included in the analyses (Figure 3). The data supports previous morphological analyses in grouping these individuals as one species with a unique evolutionary trajectory. The

high bootstrap values also support the monophyly of the species (Figure 3). The high CI and RI values indicate minimal amounts of homoplasy within the dataset (Figure 3).

It has been shown that several Testudine species consistently display low genetic variability and divergence among mitochondrial DNA lineages (Avise et al., 1992). Within the family Emydidae, *Malaclemys terrapin* populations displayed nucleotide diversity values of 0.0002 (Avise et al., 1998) indicating that the nucleotide diversity value of 0.001 in *Pseudemys gorzugi* is similar to other emydid species. Avise et al. 1992, using data from 74 mitochondrial restriction sites restriction for 53 individuals, found only six haplotypes. Of those haplotypes, four were unique to single individuals and each haplotype only differed from the others by one or two site changes (Avise et al., 1992). Those authors offered as one possible explanation for the reduced levels of genetic variation, that the *Malaclemys terrapin* populations have been in recent evolutionary contact thus homogenizing the populations. Similar results were obtained for 55 *Trachemys scripta* specimens (Avise et al., 1992). Only two mitochondrial haplotypes, differing at three restriction sites were observed (Avise et al., 1992). Again, using 59 restriction sites for 12 individual *Graptemys geographica* revealed no site variation (Avise et al., 1992).

While the low levels of mitochondrial differentiation among turtle populations is not fully understood, multiple explanations have been proposed to account for the lower nucleotide substitution rates. One possible explanation is that turtles have developed more efficient enzymes for mitochondrial DNA replication and repair mechanisms, thus resulting in reduced rates of sequence evolution (Avise et al., 1992). In addition, several correlations have been made between the reduced rate of mitochondrial DNA evolution

in turtles and the life history of these organisms. Most Testudine species have a long generation time, and it is postulated that evolutionary rates in nuclear DNA may be influenced by a generation-time effect that would decrease the amount of sequence variation in organisms with longer generations due to the reduction in the number of DNA replications per year in the germ-line (Avise et al., 1992). Another interesting correlation with the reduced rate of mitochondrial DNA evolution is the slow metabolic rate of turtles. Correlations between metabolic rate and mitochondrial evolution rate have been demonstrated in sharks and some mammals (Martin and Palumbi, 1993).

Microsatellite DNA Analysis

The preliminary microsatellite data show little population differentiation among the sample sites investigated (Figure 5). Preliminary data reveals the presence of several common alleles shared among all of the study populations, yet unique alleles and varying allele frequencies are found among the localities (Figure 6). The clustering analysis indicates that the genetic variation present in the population can be most accurately described by grouping the study localities into three clusters (Figure 5). However, when *k* was set to three, individuals were assigned to clusters without regard to geographic origin (Table 11) thus demonstrating, from a genetic perspective, the species is homogeneous throughout the range. The range of *P. gorzugi* is completely bisected by anthropogenic dewatering in the Pecos River thus splitting the populations into north (NM) and south (TX). However, the AMOVA analysis to test the north south partitioning of genetic variation indicated that only 13.8% of the total variation occurred between these two groups (Figure 10). This analysis also supports the conclusion that the

population is genetically homogenous. Additional loci should be used to further investigate these populations and hopefully better resolve the population structure of *Pseudemys gorzugi* throughout its range in Texas and New Mexico with cumulative genetic data.

Researchers have successfully amplified polymorphic loci across both marine and freshwater turtle species, demonstrating the retention of microsatellite flanking regions over approximately 3 million generations (FitzSimmons et al., 1995). It is possible that the same mechanisms responsible for the slow rate of nucleotide substitution in mitochondrial DNA is also acting on these nuclear sequences (FitzSimmons et al., 1995).

Microsatellite analyses, similar to those completed for this project, are also being conducted to evaluate the effects of a population bottleneck in one study population caused by pet trade over-collection. A previously surveyed locality with an abundance of *Pseudemys gorzugi* was almost completely extirpated in the mid-1990's but has since been partially repopulated; hopefully the results from the population bottleneck analyses will give us an indication of how the population was reestablished. That information will assist management authorities by contributing ideas about possible recovery strategies for the portions of *Pseudemys gorzugi* historical range that are currently not occupied based on the revealed natural repopulation or colonization of this particular study site. Although the statewide genetic structure in *Pseudemys gorzugi* appears to be homogeneous, qualitative allelic differences exist between even adjacent localities. Additional loci and subsequent analysis of these differences may provide evidence about the process of recolonization in these river drainage systems and thus provide predictions regarding the reestablishment of *P. gorzugi* in areas currently extirpated.

Conclusions

As previously noted, the anthropogenic changes to west Texas river systems have degraded the habitat to the point where some local populations of *Pseudemys gorzugi* are likely at considerable and continuing risk of extirpation. Surveys indicate low population densities in all localities, especially in the number of juvenile and sub-adult turtles, and thus the populations may not be able to persist without intervention. Decreased river flow rates and diminishing water quality throughout the entire range of this species makes habitat protection and restoration a vital portion of any effective management strategy for the protection of *Pseudemys gorzugi.* The work completed thus far (Bailey et al., in review; Bailey et al., in press; Bailey et al., in submission; Bailey and Forstner, 2004; Forstner et al., 2004; Stout et al., in press) and the continuing research on the life history, ecology, and abiotic factors affecting *Pseudemys gorzugi* will provide other researchers and state management authorities with up to date information on the species and the issues it faces in the desert rivers of Texas. The assembled information will eventually aid in the conservation of this unique Texas reptile.

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Figure 1. A male *Pseudemys gorzugi* collected in Val Verde County, Texas in 2004. The ventral view shows the presence of reticulate melanism (RM) on the marginal plastral scutes; the marginal scutes remain bright red in color but have invasive black vermiculations. The central plastral scutes do not show evidence of reticulate melanism and thus remain cream in color. The carapacial pattern is the same as the pattern seen in the marginal scutes; the pattern is virtually identical to the RM carapacial pattern found *Chrysemys* and presented as a photographic figure in Ultsch, 1999.

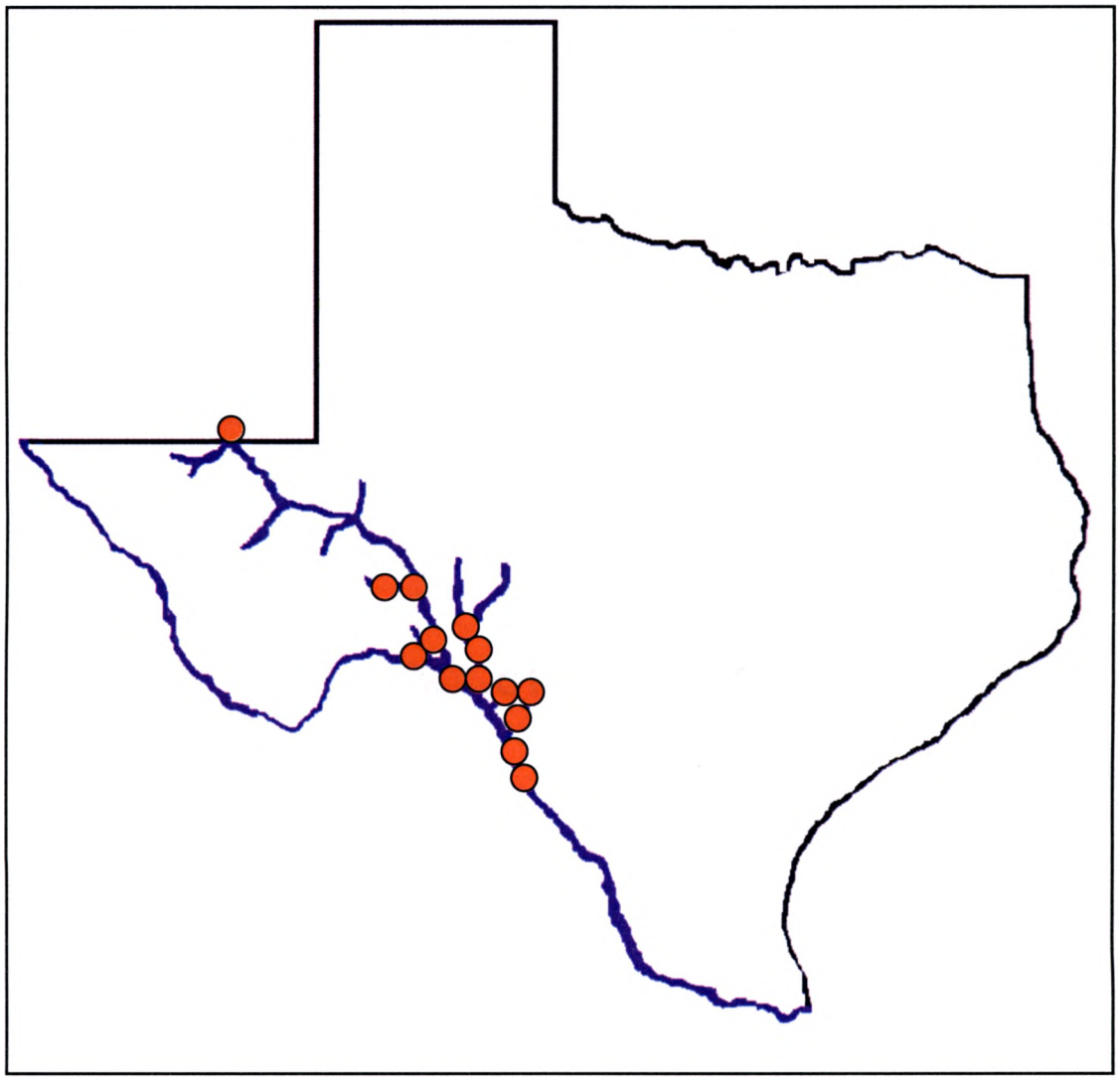

Figure 2. The map shows both the historical range of *Pseudemys gorzugi* in Texas (Blue) as well as the locations where *Pseudemys gorzugi* is currently present (Red). The actual range is considerably smaller than the presumed historical range of the species. The range contraction is believed to be a direct consequence of anthropogenic changes to the Texas landscape rendering certain areas of the potential range inhabitable by the species.

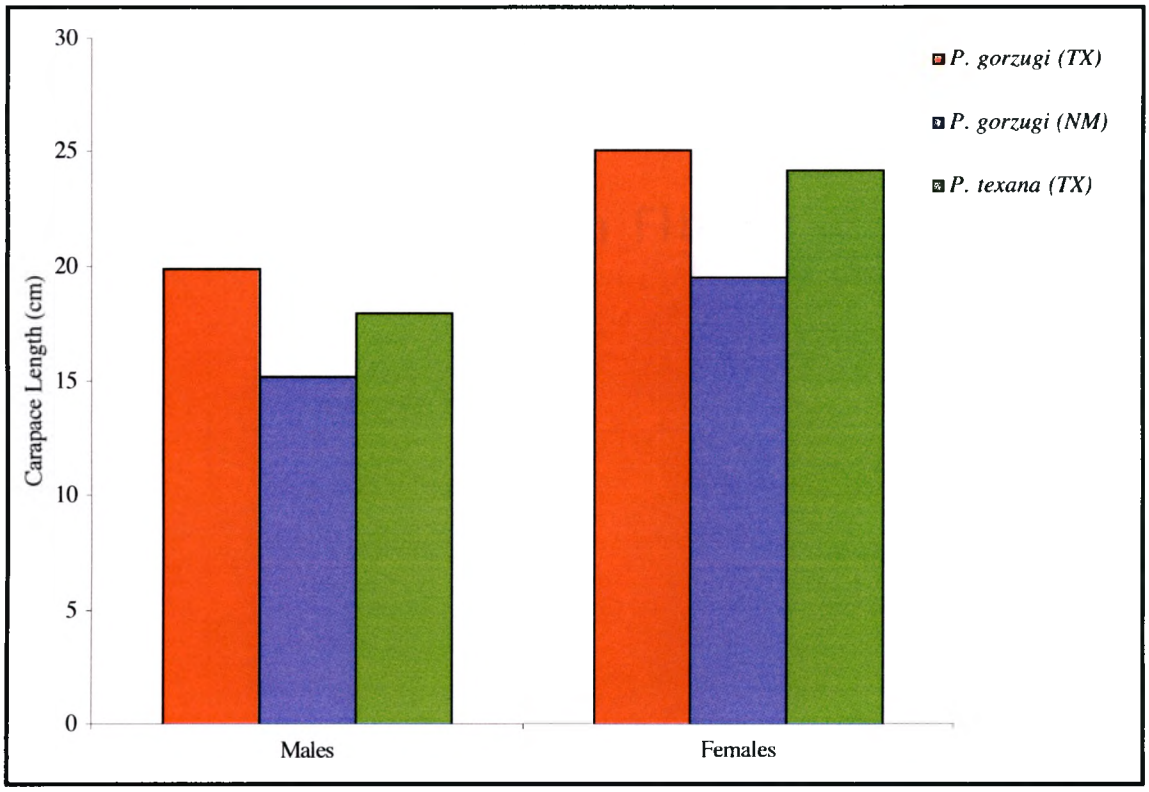

Figure 3. The histogram illustrates the size differences (carapace length; cm) for *Pseudemys gorzugi* samples from Texas and New Mexico (Degenhardt et al., 1996), and *Pseudemys texana* samples from Texas (San Marcos River). The data for both males and females is shown. The measurements are statistically different among males and among females.

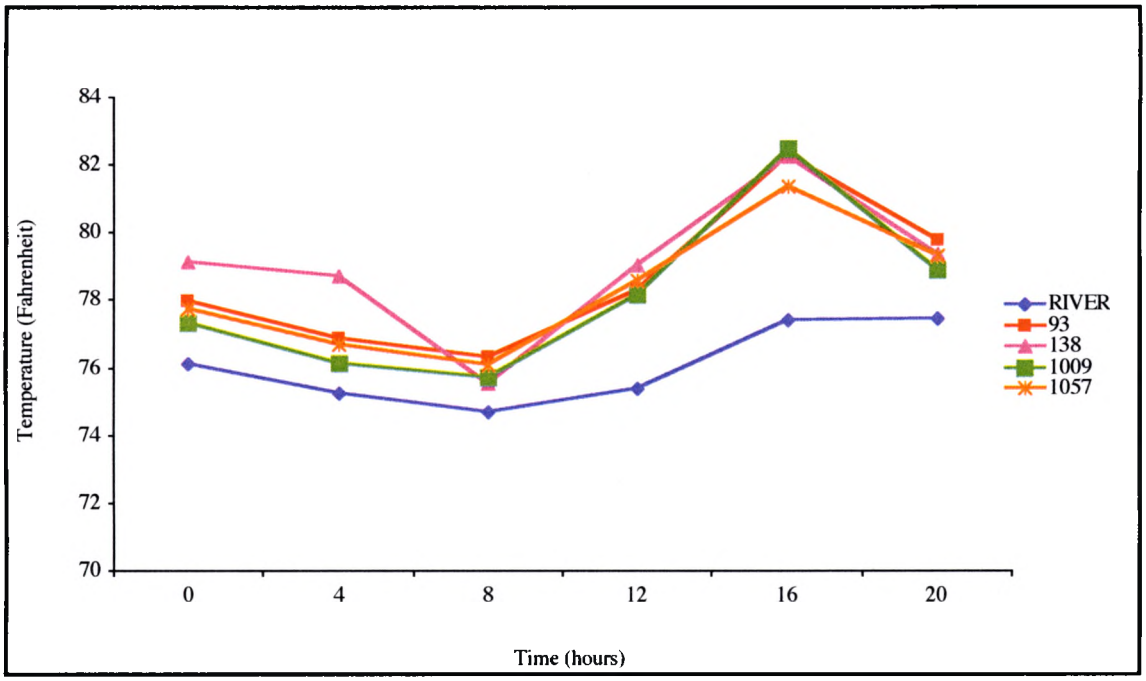

Figure 4. Summary of the Thermocron iButton temperature data obtained from four *Pseudemys gorzugi* (individuals marked 93, 138, 1009, and 1057) and the environmental sampling thermocron temperature data for the logger placed at a depth of 1M in the river. Each line on the graph represents a summary of the temperatures logged over three months during the summer of 2004. Error bars have been removed for presentation; obviously, for any given individual the daily cycle varied, but all individuals followed the same general trend across the study period.

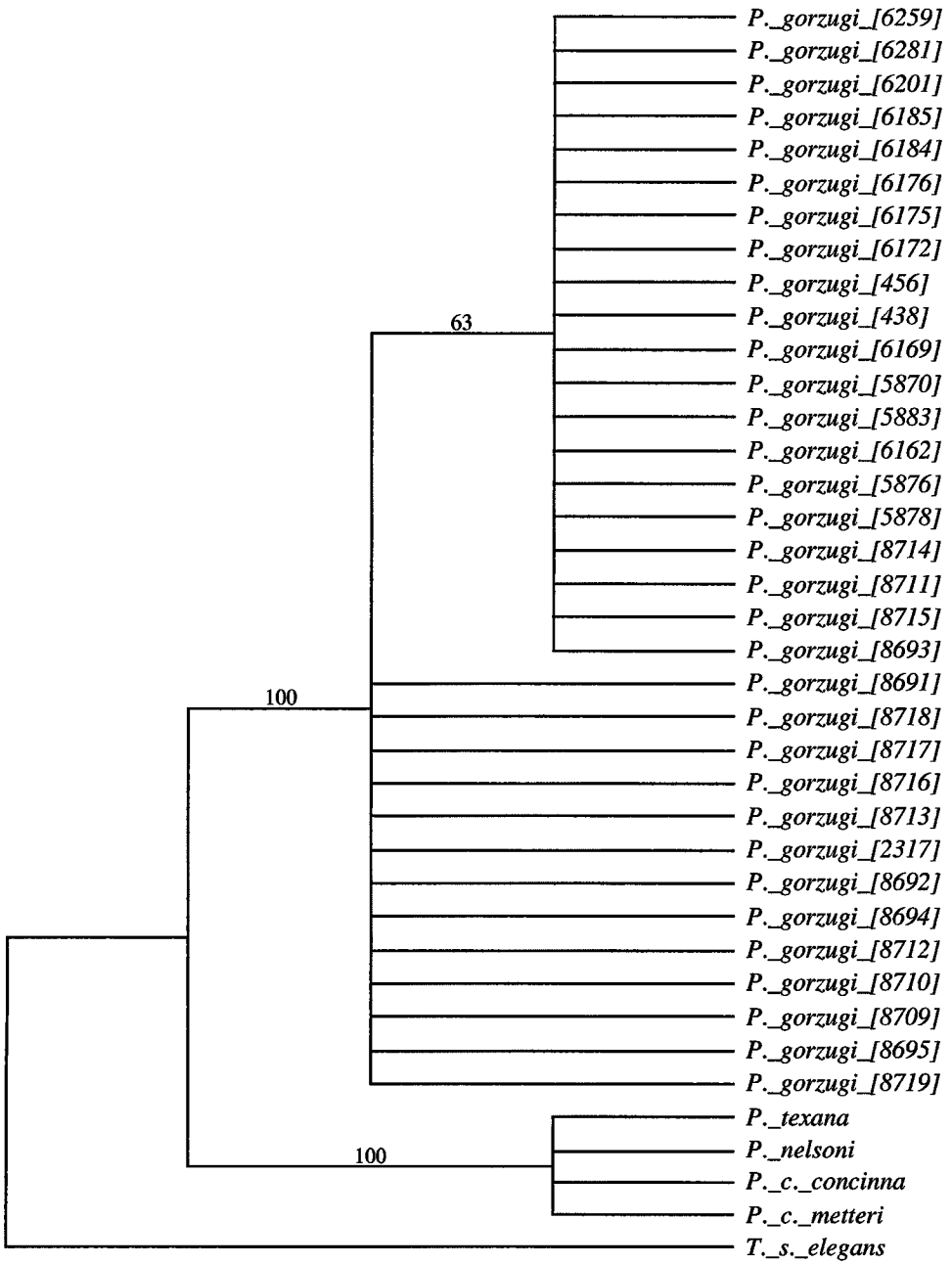

Figure 5. The most parsimonious tree discovered using PAUP for *Pseudemys gorzugi*. The bootstrap values are shown on the branches. The tree is based on 909 base pairs of mtDNA sequence data. *Trachemys scripta elegans* was used as the outgroup. The tree required 83 steps (CI=0.988, RI=0.975). Reference numbers are listed for each individual *P. gorzugi*; see Appendix 4 for specific locality information for each individual.

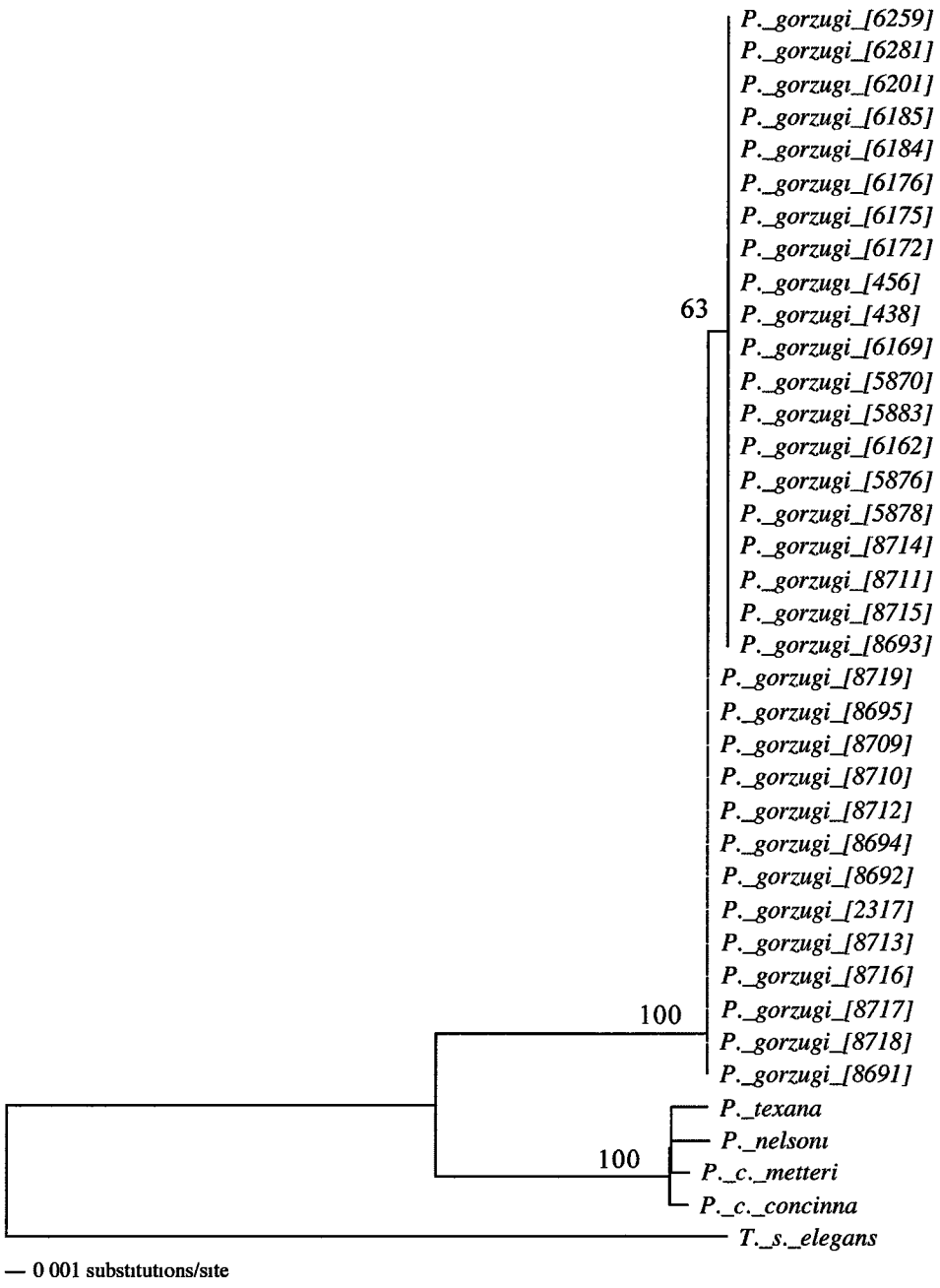

Figure 6. The neighbor-joining phylogram for *Pseudemys gorzugi* mtDNA (909 bp) using the Jukes-Cantor distance correction method. *Trachemys scripta elegans* was used as the outgroup. The bootstrap values are shown on the branches.

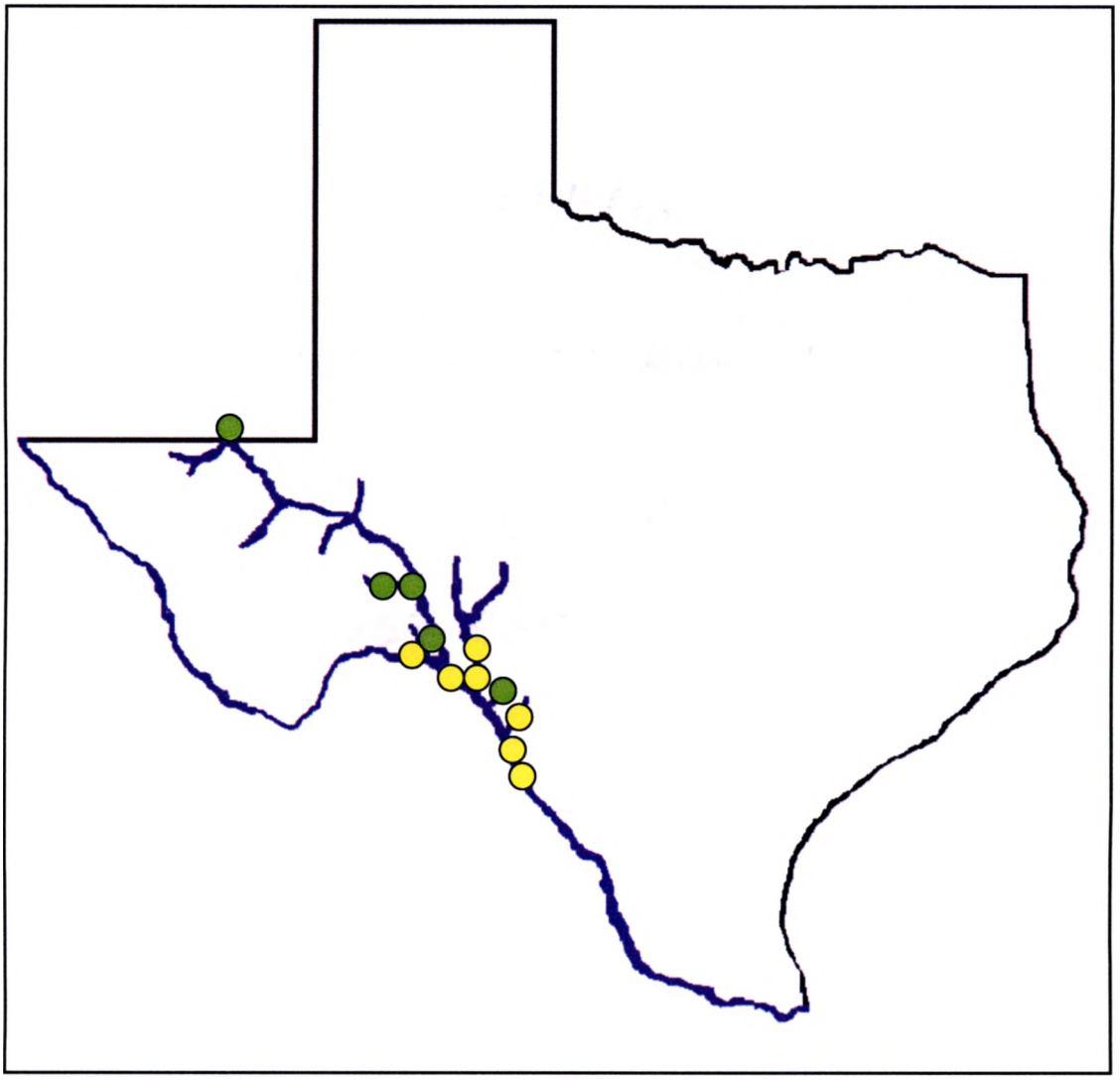

Figure 7. The map shows the results of the SAMOVA using the mitochondrial DNA for *Pseudemys gorzugi* in Texas. When k was set at 2 the Φ_{CT} was 0.69984 ($p = 0.00196$) indicating that almost 70% of the variation among the groups can be explained by placing populations into these two clusters (Green versus Yellow).

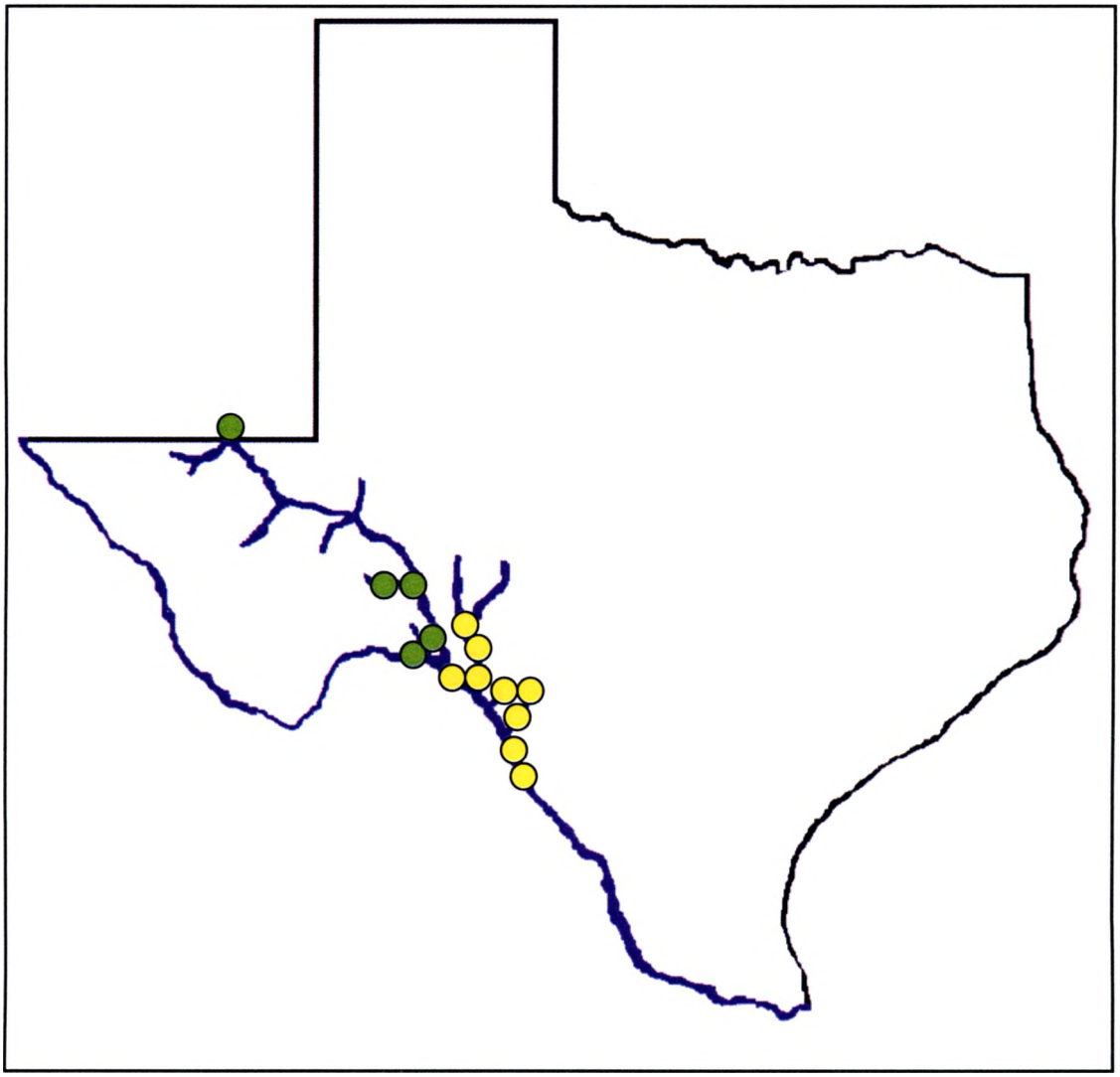

Figure 8. The map shows the results of the AMOVA using the microsatellite DNA for *Pseudemys gorzugi* in Texas. When *k* was set at 2 the Φ_{CT} was 0.13823 (p = 0.01271) indicating that only13.8% of the variation among the groups is explained by placing populations into these two clusters (Green versus Yellow).

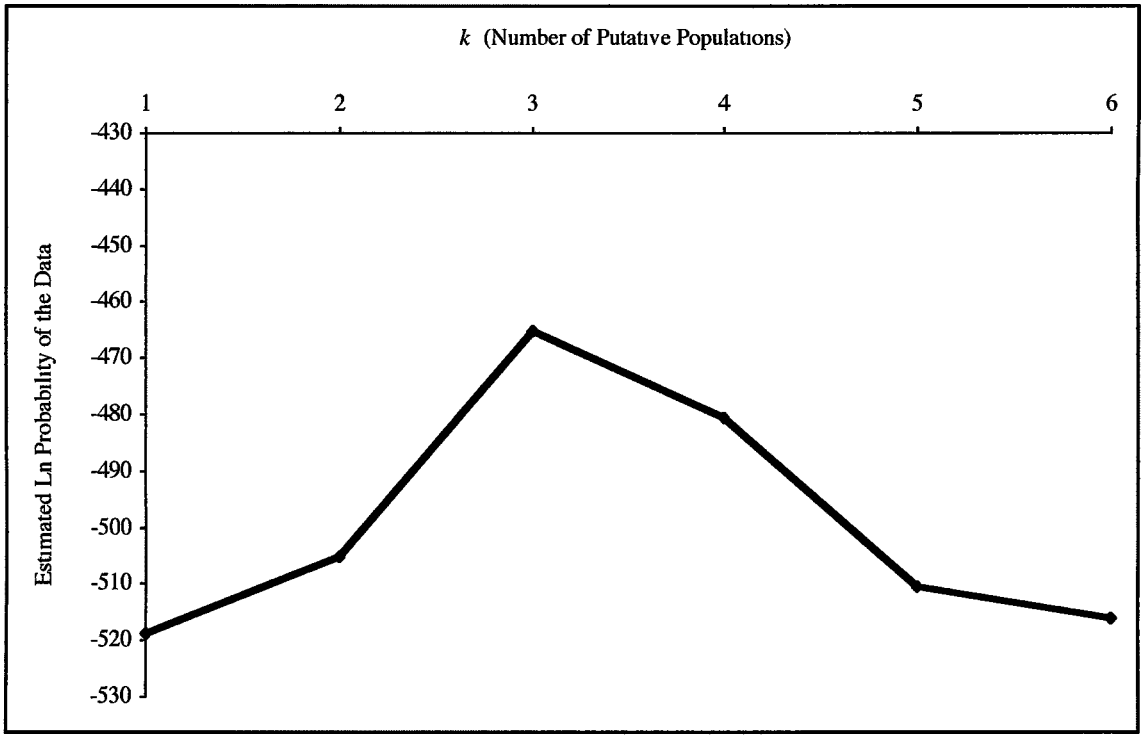

Figure 9. The figure shows the graph of the estimated Ln probability for the microsatellite data versus the number of populations for the *Pseudemys gorzugi* sampled. This graph was produced using STRUCTURE. Analyses were conducted with a burnin of 50,000 and a markov chain of 500,000 using a model allowing for admixture. Note that the graph essentially asymptotes at k=3 indicating the variation in the dataset can best be accounted for by partitioning individuals into 3 clusters.

Table 1. The number of *Pseudemys gorzugi* sample collected from each of the fourteen samples sites. Data was not available for every category at every location.

Study Sight Location	Males	Females	Juveniles	Total Number of Individuals
Eddy County 1				8
Kinney County 1	3	1		5
Kinney County 2	1	2		3
Maverick County 1	2		2	4
Maverick County 2	1	1		2
Terrell County 1	12	10		23
Terrell County 2				2
Val Verde County 1		1		15
Val Verde County 2		1		2
Val Verde County 3	62	24	1	97
Val Verde County 4		2		2
Val Verde County 5		1		1
Val Verde County 6				2
Val Verde County 7	9	11	3	23
Total	90	54	6	189

Table 2. The population meristics of the *Pseudemys gorzugi* sample collected in Texas and New Mexico. The measurements for carapace length (CL), carapace width (CW), plastron length (PL), plastron width (PW), and body depth (BD) are given in centimeters; the measurements for weight are given in grams. The mean population meristics for *Pseudemys texana* in the San Marcos River are also provided for comparison.

	CL	CW	PL	PW	BD	Weight
Male *P. gorzugi* (TX)	19.9	14.7	17.0	11.2	7.1	982
Male *P. gorzugi* (NM)	15.2					496
Male *P. texana* (TX)	18.0	13.7	15.6			762
Female *P. gorzugi* (TX)	25.1	18.9	22.6	14.6	9.9	2097
Female *P. gorzugi* (NM)	19.5					1139
Female *P. texana* (TX)	24.2	18.1	21.7			2142
Juvenile *P. gorzugi* (TX)	8.4	7.9	7.8	5.6	4.0	110
Minimum *P. gorzugi* (TX)	6.7	6.0	5.9	4.9	3.4	60
Maximum *P. gorzugi* (TX)	37.2	26.0	33.5	20.4	15.0	5600

Table 3. The table shows the measurements taken from *Pseudemys gorzugi* hatchlings bred in captivity. Measurements are given in millimeters and weight is in grams. CL stands for carapace length, CW for carapace width, PL for plastron length, PW for plastron width, and BD for body depth. The mean is given for the shell measurements.

Individual	CL	CW	PL	PW	BD	Weight
1	41.5	39.4	39.0	26.1	21.4	16.0
2	41.4	38.9	37.9	30.3	20.6	16.0
3	40.0	36.8	37.5	30.6	21.4	15.0
4	40.6	36.4	38.0	27.5	19.4	15.0
5	41.7	38.2	38.4	31.3	22.0	16.0
6	41.1	38.4	38.0	30.6	20.9	16.0
7	38.4	36.6	36.5	30.1	21.1	13.0
8	42.4	39.7	39.5	32.6	21.0	18.0
9	40.1	37.8	38.1	29.8	21.1	16.0
10	40.2	37.5	37.1	29.5	21.4	15.0
11	39.9	37.9	37.3	29.7	21.1	15.0
12	39.1	35.5	36.3	28.2	19.1	12.0
13	34.6	33.2	32.6	24.4	16.3	8.0
14	36.6	34.7	35.0	29.0	17.3	10.0
Mean	36.9	37.2	37.2	29.3	20.3	13.4
Mean for *Pseudemys texana* (N=62)	39.0	37.2	37.3			14.0

Table 4. The density of *Pseudemys gorzugi* (per river mile) in the Devils and Pecos Rivers and the density of *Pseudemys texana* (per river mile) in the San Marcos River based on observational (sightings) data.

Species	River (River miles)	Adults	Juveniles	Total	Individuals per river mile
Pseudemys gorzugi	Pecos (60)	123	3	126	2.10
	Devils (22)	68	3	71	1.18
Pseudemys texana	San Marcos (5.5)	56	16	72	13.09
	San Marcos (5.5)	77	17	94	17.09
	San Marcos (5.5)	109	19	128	23.27

Table 5. The percent dietary composition resulting from the fecal analysis of five *Pseudemys gorzugi*. The total percent composition is calculated relative to all of the individuals.

Individual	Arthropods	Crustaceans	Algae	Macrophytic Algae	Macrophytic Vascular Plants
1	5.0 %	0.0 %	60.0 %	15.0 %	20.0 %
2	16.7 %	8.3 %	33.3 %	25.0 %	16.7 %
3	4.08 %	2.05 %	85.71 %	0.0 %	8.16 %
4	6.25 %	0.0 %	78.13 %	6.25 %	9.37 %
5	11.11 %	0.0 %	55.55 %	5.56 %	27.78 %
Total Composition	6.97 %	0.96 %	69.66 %	7.47 %	14.94 %

Table 6. The observed and expected allele frequencies for each of the 14 populations at the Pseud 5 locus. No data was recorded if the locus was monomorphic in a given population.

Population	Number of Alleles	Observed Heterozygosity	Expected Heterozygosity	P Value
Eddy County 1	1			
Kinney County 1	2	0.200	0.3778	1.000
Kinney County 2	2	0.333	0.333	1.000
Maverick County 1	1			
Maverick County 2	0			
Terrell County 1	1			
Terrell County 2	2	0.500	0.833	1.000
Val Verde County 1	1			
Val Verde County 2	1			
Val Verde County 3	2	0.500	0.500	1.000
Val Verde County 4	1			
Val Verde County 5	1			
Val Verde County 6	2	0.500	0.833	1.000
Val Verde County 7	0			

Table 7. The observed and expected allele frequencies for each of the 14 populations at the Pseud 4-128 locus. No data was recorded if the locus was monomorphic in a given population.

Population	Number of Alleles	Observed Heterozygosity	Expected Heterozygosity	P Value
Eddy County 1	2	0.143	0.275	1.000
Kinney County 1	4	0.40000	0.66667	0.32407
Kinney County 2	5	1.000	0.933	1.000
Maverick County 1	2	0.333	0.600	1.000
Maverick County 2	3	1.000	0.833	1.000
Terrell County 1	3	0.158	0.152	1.000
Terrell County 2	1			
Val Verde County 1	6	0.57143	0.76923	0.551490
Val Verde County 2	0			
Val Verde County 3	1			
Val Verde County 4	10	0.636	0.792	0.547
Val Verde County 5	2	0.000	1.000	0.332
Val Verde County 6	2	0.500	0.500	1.000
Val Verde County 7	3	0.500	1.000	0.333

Table 8. The observed and expected allele frequencies for each of the 14 populations at the Galap 3 locus. No data was recorded if the locus was monomorphic in a given population.

Population	Number of Alleles	Observed Heterozygosity	Expected Heterozygosity	P Value
Eddy County 1	2	0.200	0.733	0.365
Kinney County 1	2	0.600	0.556	1.000
Kinney County 2	1			
Maverick County 1	2	0.333	0.600	1.000
Maverick County 2	1			
Terrell County 1	2	0.000	0.395	0.001
Terrell County 2	1			
Val Verde County 1	2	0.182	0.6062	0.0642
Val Verde County 2	1			
Val Verde County 3	1			
Val Verde County 4	2	0.167	0.621	0.154
Val Verde County 5	2	0.500	0.500	1.000
Val Verde County 6	2	0.000	1.000	0.334
Val Verde County 7	2	0.000	0.601	0.005

Table 9. The observed and expected allele frequencies for each of the 14 populations at the Galap 9 locus. No data was recorded if the locus was monomorphic in a given population.

Population	Number of Alleles	Observed Heterozygosity	Expected Heterozygosity	P Value
Eddy County 1	3	0.600	0.511	1.000
Kinney County 1	3	0.500	0.464	1.000
Kinney County 2	3	1.000	0.833	1.000
Maverick County 1	1			
Maverick County 2	2	0.500	0.500	1.000
Terrell County 1	2	0.222	0.464	1.000
Terrell County 2	2	0.500	0.833	1.000
Val Verde County 1	3	1.000	0.733	1.000
Val Verde County 2	2	1.000	1.000	1.000
Val Verde County 3	1			
Val Verde County 4	1			
Val Verde County 5	2	0.500	0.833	1.000
Val Verde County 6	2	0.500	0.500	1.000
Val Verde County 7	0			

Table 10. The observed and expected allele frequencies for each of the 14 populations at the Galap 13 locus. No data was recorded if the locus was monomorphic in a given population.

Population	Number of Alleles	Observed Heterozygosity	Expected Heterozygosity	P Value
Eddy County 1	2	0.400	0.356	1.000
Kinney County 1	2	0.400	0.511	1.000
Kinney County 2	2	0.500	0.500	1.000
Maverick County 1	1	0.333	0.600	1.000
Maverick County 2	1			
Terrell County 1	3	0.333	0.674	0.012
Terrell County 2	2	0.500	0.833	1.000
Val Verde County 1	3	0.100	0.674	0.001
Val Verde County 2	2	1.000	1.000	1.000
Val Verde County 3	1			
Val Verde County 4	3	0.167	0.758	0.023
Val Verde County 5	2	0.500	0.833	1.000
Val Verde County 6	1			
Val Verde County 7	1			

Table 11. The proportion of individuals that were placed in each cluster when *k* (the number of populations) was set to 3. The table is based upon the results of the STRUCTURE analysis of the microsatellite data for the *Pseudemys gorzugi* samples. The data shows no significant difference among the four populations.

Putative Population	Cluster 1	Cluster 2	Cluster 3	Number of Individuals
Val Verde County 1	0.490	0.192	0.318	14
Kinney County 1	0.033	0.543	0.424	5
Val Verde County 2	0.035	0.431	0.534	1
Terrell County 2	0.228	0.563	0.209	19
Kinney County 2	0.111	0.176	0.713	3
Val Verde County 5	0.028	0.477	0.495	2
Eddy County 1	0.125	0.613	0.262	7
Terrell County 2	0.034	0.632	0.334	2
Val Verde County 3	0.033	0.715	0.253	2
Val Verde County 4	0.352	0.284	0.364	16
Val Verde County 6	0.042	0.474	0.483	2
Maverick County 1	0.044	0.590	0.365	3
Maverick County 2	0.051	0.399	0.550	2
Val Verde County 7	0.835	0.049	0.116	12

Appendix 1. The following is a summary of recent evidence of *Pseudemys gorzugi* collected for the pet trade. This evidence supports the researchers decision to eliminate disclosure of specific study site locations from this report and helps establish the immediate need for habitat conservation in order to protect the natural populations of Pseudemys gorzugi in Texas.

1. **Rio Grande River Cooter (*Pseudemys concinna gorzugi*) Very Few In The Pet Trade!**
Posted by Excell Exotics (Contact Me!) on April 11, 2004 at 18:06:19
Registered PetHobbyist User since 2003-10-09
I have a few baby Rio Grande River Cooters (P. gorzugi), these are very hard to get in the pet trade. I have these priced at $50/each. Two or more $45/each.
Prices do not include shipping.
We accept Visa, Master Card, American Express, Discover, and E-check through Paypal.
Please send a fax or email for information or orders, along with your zip code
Excell Exotics
All turtles under 4" are for scientific and educational purposes only.
No questions asked.
http://www.excellexotics.com

2. **ADULT RIO GRANDE RIVER COOTERS**-some important information
Miami, Fl
One pair of Rio Grande River Cooters (*P. gorzugi*) $300.00 value, about 10-14inches for
P.S. PLEASE ONLY LEGAL SPECIMENS OF SPOTTEDS!! I have legal turtles; I want legal turtles in return.

3. **ALL Rio Grande Cooters have been sold!**
South Texas
Posted by Hudson Turner (Contact Me!) on April 14, 2004 at 21:12:56
Registered PetHobbyist User since 2003-05-03
Thanks to all who inquired---hopefully I will have more turtles in the near future....
Thank you Kingsnake and Benjamin for your purchase.... Earl H. Turner

Appendix 2. The appendix summarizes research conducted on *Pseudemys gorzugi* prior to the start of the current study. This information was used as a basis for determining the mode of investigation for the current project as well as a baseline for determining population status and composition.

1989: During the summer of 1989 during fieldwork in Val Verde County, Forstner locates multiple individuals at several locations and one of those is collected and vouchered at SRSU. Densities are such that without SCUBA gear more individuals are seen than can be collected while surface diving.

1990 - 1991: several localities are found that have large numbers of adults, sub adults, and juveniles observed and caught/released. I would guess that 40 adults were seen within a period of two hours in clear waters at three sites more or less continuously during the summers of these two years. No attempt was made to further qualify this as the observations themselves were while snorkeling or swimming to cool off during work done for a *C. l. lepidus* study.

1992: First trip specifically to obtain *P. gorzugi* is undertaken as part of a broader emydid DNA study. In this single day on-site many (25+) adults and sub adults are seen, a single large female is captured. A good friend and colleague now with TPWD, Mark Lockwood, working at Kickapoo caverns at the time provides good detail as to abundance and densities. The animals are actually probably overly abundance given the size of most of the habitat areas, but the microhabitat is very productive in food plants. 1994: In a survey of just one site with Dixon and Forstner were able to collect, bleed, and release 20 individuals in under an hour. At a second site the animals were likewise and abundant with several additional animals bled and released. Two individuals were retained for captive observation under the care of Dr. Scott Davis at this time. Drs. Davis and Forstner gave presentations at the ASIH/HL meetings in Athens which made mention of the unique mtDNA status of *P. gorzugi*.

1998: Alongside the survey of *Trachemys gaigeae* I visited each of the former *P. gorzugi* sites to collect additional blood samples. Small differences had been found in the mtDNA between two of the sites, which lie less than 100 miles apart. This is very uncommon among range wide *Pseudemys* samples. Our goal was to provide the samples for a fuller examination using nuclear markers. The previous sites of observation and collection provided no *Pseudemys*. This was previous to the large flood striking Del Rio in the later part of the summer of 1998. The sites were undisturbed save the lack of turtles. Tra*chemys scripta elegans* were seen, collected, and bled. A single *P. gorzugi* sub adult was collected by funnel trap in the Rio Grande. It was retained and remains in captivity.

1999: I sought data on reasons underlying the losses (supposed) to the populations. I learned from Robert Guthrie (Guthrie turtle farms) a collaborator, supporter, and friend of Drs. Davis and Forstner, that he had heard of and seen *P. gorzugi* actually offered for sale for the first time in 1996-1997. Adults were valued at $40 wholesale. We do not know if this is a connected issue but it increased my concerns.

Appendix 2. Continued.

2000: Forstner revisited the study sites (which now these localities have indeed become). In a location, which produced 20 turtles in an hour in 1994, I was able to collect a male and a female, which were forwarded to the Ft. Worth Zoo for captive husbandry and eventual propagation. It took an exhaustive 6-hour search to locate the two most wary *P. gorzugi* I had ever seen up to that time.

2001: Dixon and Forstner surveyed most of the range of *P. gorzugi* in Texas. In the days spent in the field we were successful at several locations. We were able to return with approximately 20 additional blood samples with a much broader geographic composition than the samples previously available. As part of that survey we revisited old sites, finding 8 adults in a previously virtually extirpated site. Unfortunately a separate site that had remained relatively intact with dozens of individuals seen now had very few animals. A total of 9 individuals were seen and 3 of those were sampled.

Appendix 3. Mitochondrial DNA Haplotypes. The sequence data for the two unique mitochondrial DNA haplotypes observed in *Pseudemys gorzugi* are given below. A total of 909 bases resulted from the sequencing of the ND4 gene region; analysis of the mitochondrial DNA sequence revealed one polymorphic site resolving two haplotypes; at position 360, there was a transistion between C and T.

Haplotype 1.

TGACTACCAAAAGCTCATGTAGAAGCCCCAATCGCAGGGTCAATAATCCTAG
CAGCAGTACTACTCAAACTAGGGGGATATGGAATCATCCGAATTATACCAAC
ACTAAATCCTCTATCAAAAACACTTTCCTACCCATTCATAGTACTAGCATTAT
GAGGAGTGATCATAACTGGTTCAATCTGCCTACGCCAAACAGATTTAAAATC
ATTGATTGCCTACTCATCAGTAAGTCACATAGGTCTTGTTATTGCTGCAACAC
TAACACAAACCCAATGAGCATATACAGGTGCTATTACACTTATAATCGCCCA
TGGCCTAACATCATCAATACTATTCTGCCTGGCTAATACAAACTATGAACGA
ATCCATAGCCGAACACTGTTACTAGCCCGAAACATACAACTACTATACCCAC
TAATAGGCCTATGATGACTACTCGCTAGCTTAGCCAACATAGCCATTCCACCA
ACCATTAATCTAATAGGAGAACTAACTATTATCGCCTCACTATTCAACTGATC
AAACATTACAATCCTAGCAACAGGATCGGGGACCATTATCACTGCTACATAT
ACCCTATATATGTTATCCACAACACAATGAGGAGGGAATACCCTCATATATC
AAAATAATACCACCCACCCATACACGGGAACACCTCCTCATAATTCTACATA
TCCTCCCCATAATATTATTAATAATAAAACCAGGACTAATCTTAGGTACTTTT
CACTGTTAATATAGTTTTAAAACAAACATTAGACTGTGGCTCTAAAAATAGG
AGTTCAAACCTCCTTATAAACCGAGAGAGGTGATTCACAATAAGAACTGCTA
ATTCCTATACCTGAGACTAACCCCTCAGCTCCCTCACTTTTAAAGGATAGAAG
TAATCCATTGGTTTTAGAA

Appendix 3. Continued.

Haplotype 2.

TGACTACCAAAAGCTCATGTAGAAGCCCCAATCGCAGGGTCAATAATCCTAG
CAGCAGTACTACTCAAACTAGGGGGATATGGAATCATCCGAATTATACCAAC
ACTAAATCCTCTATCAAAAACACTTTCCTACCCATTCATAGTACTAGCATTAT
GAGGAGTGATCATAACTGGTTCAATCTGCCTACGCCAAACAGATTTAAAATC
ATTGATTGCCTACTCATCAGTAAGTCACATAGGTCTTGTTATTGCTGCAACAC
TAACACAAACCCAATGAGCATATACAGGTGCTATTACACTTATAATCGCCCA
TGGCCTAACATCATCAATACTATTCTGCCTGGCTAATACAAACTACGAACGA
ATCCATAGCCGAACACTGTTACTAGCCCGAAACATACAACTACTATACCCAC
TAATAGGCCTATGATGACTACTCGCTAGCTTAGCCAACATAGCCATTCCACCA
ACCATTAATCTAATAGGAGAACTAACTATTATCGCCTCACTATTCAACTGATC
AAACATTACAATCCTAGCAACAGGATCGGGGACCATTATCACTGCTACATAT
ACCCTATATATGTTATCCACAACACAATGAGGAGGGAATACCCTCATATATC
AAAATAATACCACCCACCCATACACGGGAACACCTCCTCATAATTCTACATA
TCCTCCCCATAATATTATTAATAATAAAACCAGGACTAATCTTAGGTACTTTT
CACTGTTAATATAGTTTTAAAACAAACATTAGACTGTGGCTCTAAAAATAGG
AGTTCAAACCTCCTTATAAACCGAGAGAGGTGATTCACAATAAGAACTGCTA
ATTCCTATACCTGAGACTAACCCCTCAGCTCCCTCACTTTTAAAGGATAGAAG
TAATCCATTGGTTTTAGAA

Appendix 4. Mitochondrial DNA Haplotypes. The mitochondrial DNA haplotype for each individual sequenced is given below.

MF #	Study Site Location	Haplotype
438	Val Verde County 1	A
456	Val Verde County 1	A
2317	Val Verde County 5	B
5870	Terrell County 1	A
5876	Terrell County 1	A
5878	Terrell County 1	A
5883	Terrell County 1	A
6162	Val Verde County 1	A
6169	Eddy County 1	A
6172	Eddy County 1	A
6175	Eddy County 1	A
6176	Eddy County 1	A
6184	Terrell County 1	A
6185	Terrell County 1	A
6201	Terrell County 1	A
6259	Val Verde County 6	A
6281	Terrell County 2	A
8691	Val Verde County 5	B
8692	Val Verde County 3	B
8693	Val Verde County 3	A
8694	Val Verde County 3	B
8695	Val Verde County 3	B
8709	Kinney County 1	B
8710	Kinney County 1	B
8711	Kinney County 1	A
8712	Kinney County 1	B
8713	Val Verde County 4	B
8714	Val Verde County 4	A
8715	Maverick County 1	A
8716	Maverick County 1	B
8717	Maverick County 2	B
8718	Maverick County 2	B
8719	Maverick County 1	B

Appendix 5. Microsatellite Primers. The following is the sequence data for the five microsatellite loci investigated in the study. The forward and reverse primers are listed.

Primer Name	Primer Sequence (5' – 3')
Pseud 4-128 F	GCA AGG CTG CAC AAA CTC TC
Pseud 4-128 R	GCA GGT GTC CAC ATT GAC TTG
Pseud 5 F	TGT ACT GCA ACC AAA GAA AAT TAC
Pseud 5 R	CAT TAG AAA AGA TGA CCA AGA AAC
Galap 3 F	AGG CAA AGC ACC TGC AAA TC
Galap 3 R	CGT GTG TTT GGA CAG AAG AGT AAC
Galap 9 F	CCC TGC TGA CGA ACG ATA TG
Galap 9 R	TCT GGC ATT TCC TAG CTC TTG
Galap 13 (809) F	CCT AAC TAG AGT CTG TAA GGA AGG TAG G
Galap 13 (809) R	CCA CTC CAG AGT TGT GAC ATC AG

Appendix 6. Microsatellite Data. The following table provides the microsatallite DNA alleles called for each individual at all five loci investigated. Individuals are separated by specific study location. ? is used to denote missing data.

MF #	Pseud 5	Pseud 4-128	Galap 3	Galap 9	Galap 13
MF 438	161	222	168	130	116
	161	222	168	146	116
MF 439	161	222	168	?	124
	161	304	168	?	124
MF 440	?	?	166	?	98
	?	?	166	?	98
MF 442	?	?	166	?	?
	?	?	166	?	?
MF 443	?	?	166	86	116
	?	?	166	130	116
MF 445	161	222	166	?	98
	161	286	166	?	98
MF 446	161	222	168	?	?
	161	284	168	?	?
MF 447	161	194	168	?	?
	161	222	168	?	?
MF 448	161	278	?	?	?
	161	278	?	?	?
MF 455	?	?	?	?	98
	?	?	?	?	98
MF 457	?	?	168	?	98
	?	?	168	?	98
MF 458	?	?	166	?	98
	?	?	168	?	98
MF 459	?	222	166	?	98
	?	222	168	?	98
MF 6162	161	?	?	130	116
	161	?	?	146	124
MF 456	161	222	166	?	124
	161	222	168	?	124
MF 8709	161	222	166	130	124
	161	222	168	146	124
MF 8710	153	222	168	130	116
	161	288	168	130	124
MF 8711	161	222	166	130	124
	161	222	166	130	124
MF 8712	161	276	166	130	116
	161	300	168	164	124

Appendix 6. Continued.

MF 2317	161	?	168	130	116
	161	?	168	146	124
MF 5869	161	222	168	?	98
	161	264	168	?	98
MF 5870	161	222	168	130	124
	161	222	168	130	124
MF 5871	161	222	168	?	?
	161	222	168	?	?
MF 5875	161	222	?	?	?
	161	222	?	?	?
MF 5876	161	222	168	130	116
	161	222	168	130	124
MF 5877	?	222	?	?	?
	?	222	?	?	?
MF 5878	161	222	168	130	124
	161	222	168	130	124
MF 5879	161	222	168	?	116
	161	222	168	?	124
MF 5881	?	222	?	?	?
	?	222	?	?	?
MF 5882	?	222	168	?	98
	?	222	168	?	98
MF 5883	161	222	168	130	124
	161	222	168	130	124
MF 6184	161	222	166	130	124
	161	264	166	164	124
MF 6185	161	222	166	130	116
	161	222	166	130	124
MF 6201	161	222	168	130	116
	161	222	168	130	124
MF 8701	?	222	168	?	?
	?	266	168	?	?
MF 8702	161	222	166	130	124
	161	222	166	130	124
MF 8703	161	222	168	?	?
	161	222	168	?	?
MF 8704	161	222	?	?	?
	161	222	?	?	?
MF 8705	161	222	168	130	124
	161	222	168	164	124
MF 6159	153	276	166	130	116

Appendix 6. Continued.

	161	288	166	146	124
MF 6160	161	258	?	86	124
	161	286	?	130	124
MF 6161	161	222	?	?	?
	161	276	?	?	?
MF 6163	161	222	166	130	124
	161	222	168	130	124
MF 8691	161	280	168	130	116
	161	280	168	164	124
MF 6169	161	222	168	130	116
	161	222	168	164	124
MF 6170	161	222	166	130	124
	161	222	166	146	124
MF 6171	?	222	?	?	?
	?	222	?	?	?
MF 6172	161	222	166	130	116
	161	222	166	130	124
MF 6174	161	222	?	?	?
	161	222	?	?	?
MF 6175	161	222	166	130	124
	161	222	168	130	124
MF 6176	161	222	168	130	124
	161	266	168	146	124
MF 6199	161	222	168	130	124
	161	222	168	130	124
MF 6281	156	222	168	130	116
	161	222	168	164	124
MF 6259	156	222	168	130	124
	161	222	168	130	124
MF 6282	161	222	168	130	124
	161	222	168	130	124
MF 7380	161	?	?	?	?
	161	?	?	?	?
MF 7381	161	?	?	?	?
	161	?	?	?	?
MF 7382	161	?	?	?	?
	161	?	?	?	?
MF 7384	161	222	?	?	?
	161	276	?	?	?
MF 7385	161	?	?	?	?
	161	?	?	?	?
MF 7386	161	222	?	?	?

Appendix 6. Continued.

	161	288	?	?	?
MF 7387	?	222	?	?	?
	?	282	?	?	?
MF 7389	161	222	166	?	98
	161	270	166	?	98
MF 7390	161	222	166	?	98
	161	274	166	?	98
MF 8692	161	280	166	130	124
	161	302	166	130	124
MF 8693	161	222	166	130	116
	161	222	168	130	124
MF 8694	161	288	168	130	124
	161	288	168	130	124
MF 8695	161	222	168	130	98
	161	312	168	130	98
MF 8696	161	304	?	?	?
	161	304	?	?	?
MF 8697	161	222	?	?	?
	161	222	?	?	?
MF 8700	161	?	?	?	?
	161	?	?	?	?
MF 8713	161	222	168	130	124
	161	296	168	146	124
MF 8714	153	222	166	130	124
	161	222	166	130	124
MF 8715	?	222	166	130	124
	?	222	166	130	124
MF 8716	161	222	168	130	124
	161	278	166	130	124
MF 8719	161	222	166	130	124
	161	222	166	130	124
MF 8717	?	222	168	130	124
	?	290	168	146	124
MF 8718	?	222	168	130	124
	?	264	168	130	124
MF 10111	?	284	168	?	98
	?	298	168	?	98
MF 10113	?	296	?	?	98
	?	296	?	?	98
MF 10115	?	?	?	?	98
	?	?	?	?	98
MF 10116	?	?	166	?	98
	?	?	166	?	98

Appendix 6. Continued.

MF 10117	?	?	168	?	98
	?	?	168	?	98
MF 10118	?	?	168	?	98
	?	?	168	?	98
MF 10119	?	?	166	?	98
	?	?	166	?	98
MF 10123	?	?	?	?	98
	?	?	?	?	98
MF 10124	?	?	168	?	98
	?	?	168	?	98
MF 10125	?	?	166	?	98
	?	?	166	?	98
MF 10126	?	?	168	?	98
	?	?	168	?	98
MF 10127	?	?	168	?	98
	?	?	168	?	98

VITA

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