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Status of U.S. Populations of the Big Bend Slider (*Trachemys gaigeae*)

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Abstract—The freshwater turtle *Trachemys gaigeae* has a restricted range in the United States, inhabiting the Rio Grande drainage from central New Mexico to west Texas. This habitat has been heavily modified by man-made impoundments in New Mexico (Elephant Butte and Caballo reservoirs) and channelization in Texas (downstream of El Paso). Irrigation usage during the summer months, coupled with impacts from introduced vegetation, results in little to no instream flow from north of Las Cruces, New Mexico to approximately 160 kilometers below El Paso, Texas. This represents a total distance of nearly 480 kilometers (or approximately one third of the U.S. distribution of this turtle), that is affected before the river is replenished at its confluence with the Rio Conchos. The dramatic changes in this species' habitat, relative ignorance of its ecology, and potential for resulting population decline warranted investigation of the distribution, relative abundance, and genetic structure of the present populations of *T. gaigeae*. We surveyed the known U.S. range of *T. gaigeae* and non-consumptively collected DNA, individually marked those captures, and re-released the animals at the collection locality. We provide a directly comparable dataset for *Trachemys scripta elegans* from the Rio Grande drainage outside of the range of *T. gaigeae*. The genetic analyses of both mtDNA and nDNA markers provides a snapshot of the levels of genetic variability within and between *T. gaigeae* and *T.s. elegans* in the Rio Grande and indicates introgressive hybridization with non-native released "pet" *T. s. elegans*. Given the already compromised nature of the Rio Grande and the continuing decline of this ecosystem, careful management attention is required to prevent the extinction of *T. gaigeae*.

Introduction—Turtles in the family Emydidae are common entities in nearly all bodies of freshwater in the eastern and central United States. Frequently encountered in both the pet trade and in wild or suburban water systems, these turtles are the most widely distributed and abundant turtle group in the Western Hemisphere. While taxonomic contention exists regarding the arrangement and composition of genera within the Emydidae (e.g Seidel and Smith 1986), the so-called sliders are most commonly placed in the genus *Trachemys*. This species assemblage contains representatives that vary from the now cosmopolitan and ubiquitously introduced formly strict U.S. species, *Trachemys*

scripta elegans (the red-eared slider) to isolated and rare taxa like *T. taylori* found only in a single basin of central México.

The taxonomic contention does not stop at the generic level in this group. Many changes in taxonomic rank (species or subspecies) have been proposed concurrent with generic level revisions. Two main divisions of species groups within *Trachemys* are of special relevance to the current study: a group of North American taxa (*scripta*, *elegans*, and *troostii*) and a group of predominantly Mexican taxa (*cataspila, emolii, grayi, hartwegi, hiltoni, nebulosa, ornata, taylori, venusta,* and *yaquia*). The North American group ranges widely across the eastern U.S. and southwest to Texas and northeastern México. The Middle American taxa tend to have more restricted ranges, reflective of the vastly different climate of western México, and occur in isolated river drainages. Legler (1990) reviewed the putative evolutionary relationships and biogeography of these taxa in detail. According to a recent published taxonomic compilation (Iverson 1992; Seidel et al. 1999; Seidel 2002) a single representative of the Mexican group occurs in the United States, *T. gaigeae*. The most recent taxonomic treatment by Seidel et al (1999) concludes that it is appropriate to recognize *T. gaigeae* as a full species based on morphology. In a more recent revisions (Seidel 2002; Stuart and Ernst 2004) conclude the Nazas Slider (*T.s.harwegii*) is a subspecies of *T. gaigeae*.

The Big Bend slider (*Trachemys gaigeae*) is one of the least known North American emydid turtles. Despite a fairly long taxonomic history, its taxonomic status remains disputed. Hartweg (1939) first described *T. gaigeae* from a specimen collected in Boquillas Canyon in Brewster County, Texas. Subsequently, the taxon has been placed in three different emydid genera and seen several changes of specific rank. The nomenclature for *T. gaigeae* is summarized (drawn mostly from Smith and Smith 1979) in the following compilation:

Pseudemys scripta gaigeae Hartweg (1939)
Pseudemys gaigeae Stejneger and Barbour (1939)
Chrysemys scripta gaigeae Smith and Taylor (1966)
Pseudemys scripta gagei Ernst (1967)
Chrysemys gaigeae Weaver and Rose (1967)
Chrysemys gaigeae gaigeae Weaver and Rose (1967)
Pseudemys scripta gaigeae Degenhardt and Christiansen (1974)
Chrysemys gaigeae Ashton et al. (1976)
Chrysemys scripta gaigeae Morafka (1977)
Chrysemys gaigeae Morafka (1977)
Pseudemys scripta gaigeae Smith and Smith (1979)

Trachemys gaigene Ward (1984)
Trachemys gaigeae Dixon (1987)
Trachemys scripta gaigeae Iverson (1992)

The taxon occurs in two rivers within one basin, the Rio Conchos in México and the Rio Grande on the U.S./Mexican border (Iverson 1992). This study examined populations in the Rio Grande, however; no attempt to assess the current status of Mexican populations of *T. gaigeae* was made. The Rio Grande itself has been dramatically modified from its seasonally pulse-flooded natural state by two major impoundments, Elephant Butte Reservoir in 1916 and Caballo Reservoir in 1938, and by channelization of large portions of the drainage in both New Mexico and Texas. The impoundments have prevented upstream dispersal into New Mexico populations fragmenting the population and channelization may have affected the suitability of the river as habitat for *T. gaigeae*. The anthropogenic changes and demands on the Rio Grande have steadily increased over time and have forever altered the environment within which *T. gaigeae* exists (Fig. 1).

Aside from habitat destruction, the remnant populations of *T. gaigeae* are potentially threatened by introgression of *T. s. elegans* DNA into their populations. A native

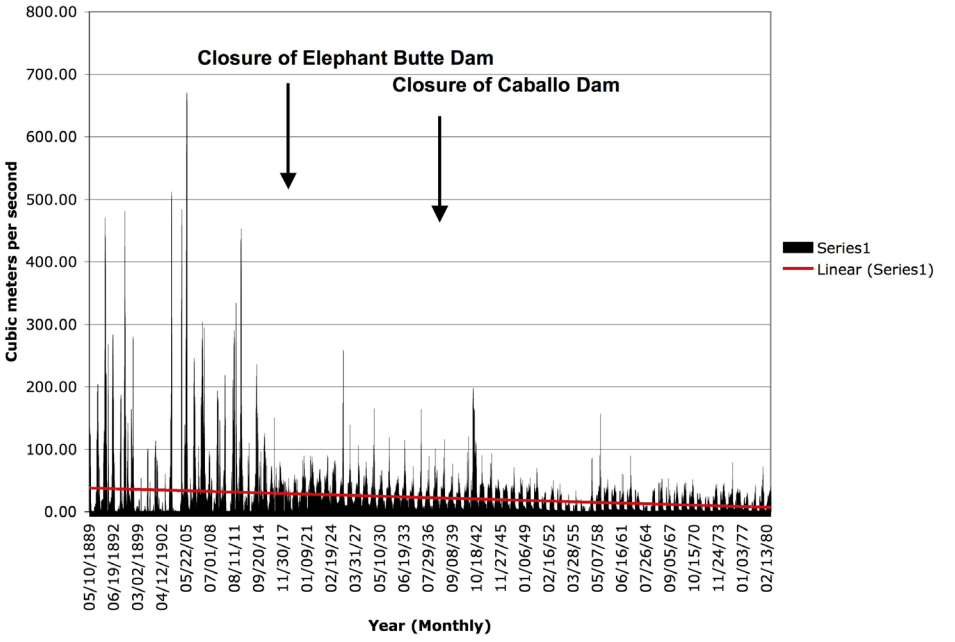

Fig. 1—Rio Grande flow at El Paso gauging station from 1889 to 1979 (Dougherty 1980) and 1980 to 1983 from the International Boundary and Water Commission. Note the periodicity of pulse flooding prior to the closure of the reservoirs. Linear regression trend line plotted across all years providing the trend over time.

population of *T. s. elegans* is found in the lower Rio Grande and ranges upstream to the southern boundary of the range of *T. gaigeae* (Legler 1990). Furthermore, introduced (non-native) *Trachemys scripta elegans* have been found in ponds along the Rio Grande, in New Mexico at Bosque del Apache National Wildlife Refuge, and within the boundaries of Big Bend National Park at Rio Grande Village. The potential for hybridization provides yet another threat to the persistence and genetic integrity of *T. gaigeae* populations.

Trachemys gaigeae is protected from collection in New Mexico, but otherwise is not currently accorded protected status at the state or federal level, and the lack of population information necessary for informed management has been previously noted (Smith and Smith 1979). The species is "Vulnerable" in the assessment of the International Union for the Conservation of Nature and Natural Resources (IUCN) (Baillie and Groombridge 1996). We sought to address part of the data deficit by field surveys to establish the current range of the species in the U.S., evaluate the systematic status of the taxon using molecular data, and to examine the environmental context of any perceived impacts on the populations in the wild. To accomplish those goals we complemented extensive field surveys with DNA marker evaluation for the samples collected in the field. In order to resolve systematic and population genetic issues, we collected genetic data from both mitochondrial DNA and the nuclear genome.

Mitochondrial DNA (mtDNA) has several properties making it useful for phylogenetic reconstruction (Harrison 1989). It is maternally inherited with a relatively high rate of nucleotide substitution. It is useful in answering questions involving the relationships between closely related species and can be a powerful tool for conservation genetics (Hillis et al. 1996). Two specific regions of the mtDNA genome have been targeted for sequence analyses of *T. gaigeae*. The first of these is a fairly conservative region containing a protein coding gene fragment and three tRNA genes. This ND4-Leu region of the mtDNA consists of the terminal 246 codons of the NADH subunit 4 gene, and the tRNAs Histidine ($tRNA^{His}$), Serine ($tRNA^{Ser}$), and Leucine ($tRNA^{Leu}$). This region has already proven itself useful in many other reptile groups (Arevalo 1992; Forstner et al. 1995; Sites et al. 1996) for elucidating relationships at and above the species level.

To examine divergence within *T. gaigeae*, the fastest evolving fragment of the mtDNA genome, the D-loop or control region (Kasamatsu et al. 1971; Clayton 1982, 1991), was also examined. This control region has been previously determined to be the most variable portion of the mitochondrial genome (Upholt and Dawid 1977; Crews et al. 1978; Brown 1983, 1985; Brown et al. 1986). It is therefore valuable for population

genetics (Stoneking et al. 1991; Baker et al. 1993) and fine scale examinations at or below the species level (Cann et al. 1987; Meyer et al. 1990; Thomas et al. 1990; Ishida et al. 1995; Parker and Kornfield 1997). It has been successfully applied to examinations in marine turtles (Norman et al. 1994; Bowen and Avise 1995; Dutton 1996) and more generally as a specifically suitable tool in conservation genetics (Schonewald-Cox et al. 1983; Taberlet 1996).

The maternal inheritance factor of mtDNA, which makes it so useful for drawing historical biogeographic inferences (Avise et al. 1987), can also be a limiting factor, as only information about the maternal parent can be garnered (Cronin 1993). To complete the genetic picture of an organism, nuclear biparentally inherited markers should also be examined. Within the nuclear DNA exist random regions of repeated sequence motifs known as microsatellites. Microsatellites are ubiquitous throughout the nuclear genome and are often highly polymorphic between species and even between individuals (Fitzsimmons et al. 1995). The degree of polymorphism increases with the number of repeat units and is due to strand slippage during DNA replication. The amount of slippage, and hence the variability of a microsatellite locus, depends on the size of the repeat as well as its base composition. Dinucleotide repeats slip more frequently than larger repeats, while sequences rich in GC repeats occur less often (Levinson and Gutman 1987). Longer microsatellites have a higher rate of slippage and are therefore usually more polymorphic. The alleles tend to differ by one or two repeat units between generations (Weber and Wong 1993) and therefore will follow a stepwise mutation model (Goldstein and Pollack 1997).

The combination of rapidly evolving mitochondrial sequences in conjunction with highly polymorphic nuclear microsatellites provides a powerful set of tools for understanding systematics and population structure. Using DNA markers as conservation tools allows us to identify evolutionarily significant units (ESU) (Moritz 1994a, 1994b; Vogler and DeSalle 1994) as well as to assess the current status of a population (Avise 1989, 1994) and thereby provide a solid foundation for management decisions.

Methods—*Field Surveys*—A survey of the Rio Grande was performed during May 1997 and from May to July 1998, at approximately 160 km intervals in New Mexico and 400 km intervals in Texas with more intensive sampling within the known current range of *T. gaigeae*. Access to the river was obtained via public access points or in coordination with private landowners. All specimens were collected alive, either by hand netting or by using hoop-net style live-traps (Anderson 1965). Captured turtles were

photographed, marked (RF tag, AVID, Inc.) (Camper and Dixon 1988), and bled from the femoral vein using 25 gauge needles and 1cc syringes. A maximum of 0.1 cc of blood was obtained from adult specimens; samples from smaller individuals were scaled down in volume accordingly. In several localities trapping was repeated in an attempt to estimate population size from recapture information. Taxonomic identification of individuals was initially based upon morphological and phenotypic characters in the field. Later assignment of pure or hybrid status was based upon the results of DNA marker analyses. We isolated individuals overnight in tanks to obtain fecal samples for determining the diet of *T. gaigeae* in the wild. Individuals were maintained in shallow water tanks for 24 hours and fecal matter was collected at presentation, preserved in 70% ethanol, labeled, and stored for subsequent examination. Material from fecal samples was identified using a dissecting microscope.

DNA marker analyses—DNA was obtained using a tissue extraction kit (Quiagen #29304) and the manufacturer's protocols. Successful extractions were judged visually by staining the DNA with ethidium bromide after electrophoresis in 1% agarose mini-gels. A 959 base pair (bp) fragment comprising the 3' terminus of the ND4 gene with the structural genes Histidine ($tRNA^{His}$), Serine ($tRNA^{Ser}$), and Leucine ($tRNA^{Leu}$) and a 421 base pair (bp) fragment of the mitochondrial D-loop were amplified using the polymerase chain reaction and suitable primers (Table 1). PCR products were checked by electrophoresis on 1% agarose gels stained with ethidium bromide. All PCR reactions were run with both positive (*Pseudemys*) and negative (no DNA) controls following Forstner et al. (1995). The fragment was prepared for sequencing using the Quiagen PCR Purification Kit (Quiagen #28106) using standard protocols modified by a final elution with 55°C sterile distilled water following 5 minutes incubation. The cleaned products were electrophoresed alongside pGEM sequencing standard (ABI #401434) in 1% high resolution agarose to assess final template concentration. The products were then cycle sequenced by the following protocol: template, primers, and water (total volume 4.5 µl) were denatured at 100°C for 2 minutes and then snap-chilled for 2 minutes on ice. Dye terminator mix (4.0 µl) was added and mixed by pipetting, after which samples were briefly centrifuged and mineral oil added to cover the reaction. PCR parameters were 25 cycles of 96°C for 30 seconds, 50°C for 1 minute, and 60°C for 4 minutes. The completed sequencing reactions were cleaned by centrifugation through 0.05g Sephadex G-50 hydrated in 800 ml of distilled water within CentriSep columns (CentriSep #PFR00105). The final elutants were dried by vacuum centrifugation and stored at -80°C prior to being analyzed on ABI 373 A and 377 XL automated DNA sequencers.

TABLE 1—DNA marker loci and synthetic oligonucleotide primer sequences used in the examination of *Trachemys gaigeae* populations in the Rio Grande drainage system.

mtDNA	Primer Name	Primer sequence 5'-3'
ND4-Leu	ND4	TGACTACCAAAAGCTCATGTAGAAGC
	Leu	TACTTTTACTTGGATTTGCACCA
	Turt1	GATCCTCTATCAAAAACACT
	Turt2	TTTTAGAGCCACAGTCTAAT
	TurtForward	TTATCCACAACACAATGA GG
	TurtReverse	TAATAATAGTGTTCGGCTATG
D-loop	DL4	TTATTTRCCACTAGCATAT
	CR2F	GTACGTACAAGTAAAACTACCGTATGCC
Nuclear Loci		
Galap3	Galap3F	AGGCAAAGCACCTGCAAATC
	Galap3R	CGTGTGTTTGGACAGAAGATGAAC
Galap4	Galap4F	GCTAAAGACCTAGTTCTGCCATG
	Galap4R	TTCAGTGGTTACTCAGCAAAGG
Pseud4-128	Pseud4-128F	GCAAGGCTGCACAAACTCTC
	Pseud4-128R	GCAGGTGTCCACATTGACTTG
Pseud225-1	Pseud225-1F	GCTTCTATGAAGATGGCTTTTTGAAC
	Pseud225-1R	TGGTCGGTGTGTTGAATTGAGGA
Pseud225-2	Pseud225-2F	TCCTCTATTCAACACACCGACCA
	Pseud225-2R	CCGCAGCATACTAATTGACTTTG
Tufu2	Tufu2F	TGCTCCTCATTATGGTACAGGGTG
	Tufu2R	TCTGCCTCTCACACACAAACTCAG

The samples were rehydrated in a 1:5 ABI loading buffer:deionized formamide solution and denatured just prior to gel electrophoresis. The 6% acrylamide gel preparation and run parameters follow the procedures outlined in the ABI User Manual. All templates were sequenced in their entirety for both strands, and printed electropherograms checked to verify accuracy of base-calling by the ABI software. Individual sequencing reactions were aligned concurrently and individual positions verified by cross-reference. Alignments of all sequences for the protein coding regions

of the ND4 gene follow published alignments (Forstner et al. 1995) and were assembled using Sequencher 3.1 (GeneCodes Corp.) using a variety of alignment criteria (Wheeler 1995).

Phylogenetic analyses of sequences utilized the program PAUP 4.0b10 (Swofford 2003). The number of taxa examined precluded the use of exact search options (Swofford 2003); therefore heuristic searches were utilized in all parsimony analyses. Multiple outgroup taxa (*Malaclemys* and *Pseudemys*) were included in the analyses. The utility of the region in the examination of interspecific and generic relationships within emydid turtles has been previously determined (Starkey et al. 2003).

After determination of the shortest tree(s), bootstrap and jack-knife analyses (2500 replicates) were utilized to determine the degree of support for each node from internal re-sampling of the data (Felsenstein 1985; Hillis and Huelsenbeck 1992; Hillis and Bull 1993; Hillis 1995). Distance measures, using corrections for nucleotide sequence data suggested by Tamura and Nei (1993), were examined using Neighbor Joining analysis in Paup 4.0b10 (Swofford 2003) and MEGA (Kumar et al., 1993).
The isolation of microsatellites in turtles followed the procedures outlined by Hillis et al. (1996) and produced 6 polymorphic loci out of a total of 32 loci isolated and characterized in our laboratories (MRJF and SKD) across a variety of turtle families (Miller 2001; Dutton 1996; Kichler 1996; Louis 1997). Primer pairs were designed in the flanking regions of each of those microsatellites and tested to determine optimal annealing temperatures. Optimized primers were end-labeled with ^{32}P-gamma ATP and used to amplify microsatellites in all individuals. Products were electrophoresed on 6% denaturing acrylamide gels and exposed to X-ray film. Alleles were scored directly from the autoradiographs. Polymorphic loci and the corresponding PCR primers are provided in Table 1. The microsatellite data were used to compare New Mexico and Texas populations of *T. gaigeae*, differences between *T. gaigeae* and *T. elegans*, and to examine population structure along the Rio Grande for *T. gaigeae*.

Results—*Field Surveys*—While efforts were made to examine the entire potential distribution of *T. gaigeae* in both Texas and New Mexico, actual survey sites were influenced by the positions of public access points along the river drainage. No new populations of *T. gaigeae* were identified by our surveys. Assuming that *T. gaigeae* historically inhabited the majority of the Rio Grande from New Mexico to west Texas, the present range is restricted to three isolated populational remnants (Fig. 2). In New Mexico *T. gaigeae* were successfully collected in Elephant Butte Reservoir and Bosque del Apache National Wildlife Refuge. Although no *T. gaigeae* were collected in or near

Caballo Reservoir the species was found there by James Stuart in the early 1990s. We did not observe nor did we capture any *T. gaigeae* in the clear fast waters below the dams. We did frequently observe both *Chrysemys picta belli* (painted turtle) and *Apalone spinifera* (spiny softshell) in those situations but no *T. gaigeae*. In Texas, *T. gaigeae* were collected from an isolated population within Hudspeth County, in Presidio County and in Brewster County. Nearly all of the current range of *T. gaigeae* in Texas is within either state (Big Bend Ranch State Park and Black Gap Wildlife Management Area) or federally (Big Bend National Park and Rio Grande Wild and Scenic River) managed lands. The species was never observed nor collected in or adjacent to channelized portions of the river. Upstream channelized sections within the agricultural communities of Tornillo and Fabens (El Paso County) failed to provide any turtles in the river drainage proper. The water depth of the river in those sections of El Paso County was uniformly less than 15 cm in all of June of 1998. In agricultural drainage systems adjacent to the river, we were able to trap *Apalone spinifera* and *Kinosternon flavescens* (yellow mud turtle) at low densities. Trapping of those areas further downstream generally failed to produce turtles. Notably, a single *Apalone spinifera* collected near Ruidosa, Presidio County, Texas was unique enough that local residents were uncertain whether this generally abundant taxon naturally occurred in the river (MRJF pers. obs.). Below the confluence of the

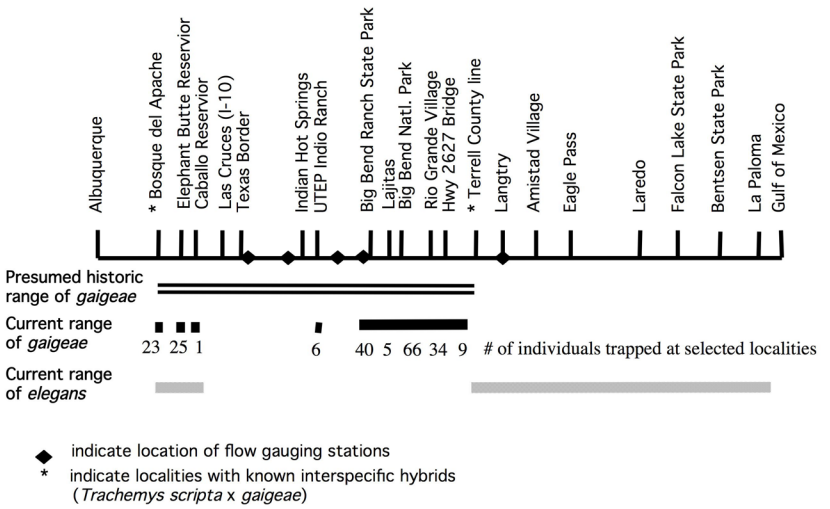

Fig. 2—The current distribution of *Trachemys gaigeae* in the Rio Grande with depiction of the presumed historical range of the taxon. Localities with known hybrid animals (based on phenotype x mtDNA haplotype disagreement) are marked with an "*". Five gauging stations are shown on the figure (El Paso, Ft. Quitman, Presidio, Alamito Creek, and Langtry). The schematic is approximately to scale.

Rio Conchos, the Rio Grande regains flow and *T. gaigeae* were present. The highest concentrations were in deeper pools immediately adjacent to riffles. Juvenile *T. gaigeae* were observed at both dawn and dusk consistently disappearing and reappearing in the downstream riffle boundaries. These animals appeared to have been feeding on riffle insects (see diet section below). This observation was corroborated throughout the sections of the river in both Brewster and eastern Presidio counties.

The Rio Grande widens, deepens, and slows considerably after passing into the lower canyons section at the eastern border of Brewster County. The numbers of *T. gaigeae* trapped and observed dropped off steadily as the character of the river changed at the Terrell County line. While the current survey was unable to physically assess most of the river from Terrell County into Val Verde County, our limited trapping in Val Verde County, historical records, and discussions with local residents indicates that *T. gaigeae* is very unlikely to range eastward beyond the Brewster-Terrell county line in the Rio Grande. Impoundment of the river at Amistad Dam has inundated the upstream section to the Langtry area. Trapping at these sites and all sites further south produced *Trachemys scripta elegans* and *Apalone spinifera* in variable abundance, and included a single *Pseudemys gorzugi* collected in the river just above Langtry.

Trapping results—We captured 169 turtles (Table 2) during a total of 67 days of trapping across two years (17 days May 1997; 15 days May; 27 days June; and 8 days July 1998). The project required more than 6,000 person hours in the field at the trapping localities. The average number of traps set per day was 12 (range 4 to 12). Traps were set for an average of 8 hours (range 4 to 24) and always checked within any 8 hour period. During the 1998 field season, 169 successes occurred for 4,500 trap hours within the range of *T. gaigeae* for an overall 4% success rate per trap set.

We collected one adult *T. elegans* at Rio Grande Village in Big Bend National Park. This is well inside what we believed to be "pure" *T. gaigeae* populations. This *T. elegans* individual was phenotypically indistinguishable from animals we have collected in Louisiana or Florida. While we have seen *T. elegans* in the Pecos River and adjacent Amistad Reservoir, the Pecos River of *T. elegans* is typically divergent in phenotype from the phenotype of the Mississippi River basin widely introduced (Seidel et al. 1999) by the pet trade. While it is possible that this individual represents a long distance dispersal from the Pecos River system, it seems more plausible that this turtle was released in the park. Unfortunately, since 1998, several more individuals have been seen within Big Bend National Park (BBNP), and in 2003 several were collected near Lajitas.

At the southern end of the range we encountered turtles with *T. elegans* phenotypes with increasing regularity and concurrently a decrease in the number of young and/or

female *T. gaigeae*. By the Brewster-Terrell county line we were collecting predominantly old, melanistic, male *T. gaigeae* and the occasional *T. elegans* phenotype. One distinguishing characteristic we noted during the surveys, subsequently supported by mtDNA data, was that adult male *T. gaigeae,* even when completely melanistic in body and carapace, retain a significant amount of yellow in the plastron, differing from the dark plastron seen in *T. elegans*. Table 2 is a summary of the trapping results for the field seasons.

The distribution of *T. gaigeae* in the Rio Grande spans approximately 362 river kilometers (Fig. 2). However, the species is not evenly distributed along that length (Fig. 2). At two sites in Brewster County we were able to re-trap a section of the river after a two-week absence. In both cases we recaptured previously tagged individuals with a high relative frequency (~20% recapture rate).

Dietary assessment—The investigation of the dietary intake of *T. gaigeae* was not a formal part of the proposed study. However, our observation of potential differential feeding by juveniles and adults led us to conduct experiments to discern the diet preferences of juveniles and adult *T. gaigeae* in Texas. Observations during the study and a collection of 40 fecal samples of Texas *T. gaigeae* provided relevant information.

Table 2—Summary of trapping results for Trachemys gaigeae in the Rio Grande drainage system during 1997 and 1998.

State	Sex	#	Mean Plastron Length (range)	Mean Plastron Width (range)	Mean Carapace Length (range)	Mean Weight (range) g
New Mexico						
	F	19	193.22 (±41.23)	120.42 (±24.91)	210.44 (±44.30	1324.00 (±650.70)
	M	27	145.15 (±24.72)	94.65 (±15.36)	164.11 (±28.13)	564.59 (±254.42)
	?	4	34.5 (±5.97)	27.75 (±4.11)	38 (±6.22)	10.90 (±5.20)
		n=50				
Texas						
	F	69	142.92 (±32.69)	95.12 (±19.97)	156.82 (±36.06)	580.98 (±360.98)
	M	83	113.51 (±25.03)	75.31 (±14.17)	128.18 (±26.52)	299.36 (±213.75)
	?	1	85	60	97	120
		n=153				

Previous evaluations have concluded that both adult and juveniles in New Mexico feed primarily on one or a few species of filamentous algae and submerged macrophytes, juveniles are more carnivorous (Stuart and Painter 2002). In Texas juveniles (those below 140 mm) feed primarily on several groups of bottom dwelling riffle insect larvae (stoneflies, mayflies, and dragonflies). Adults in Texas were found to contain primarily vegetation, primarily new shoots of common reed (*Phragmites australis*). While not all of the vegetation samples could be positively identified, *Phragmites* appears to be significantly preferred over giant river cane (*Arundo donax*) among the samples taken. Some adult individuals also contained small ants, spiders, and snails which are believed to be incidental bycatch from the ingestion of streamside cane. We also observed *T. gaigeae* scavenging fish carcasses; so while primarily herbivores, they certainly consume fish and other animal tissue if available to them.

MtDNA sequence analysis—The sequences of the mitochondrial DNA fragment of ND4-Leu provides a well supported phylogeny for *Trachemys* (Fig. 3, Fig. 4). The mtDNA sequences of the control region indicate very little differentiation in mtDNA sequences across the range of *T. gaigeae* in the U.S. In fact, bootstrap parsimony and neighbor-joining analyses of the control region sequences fail to support distinctive clades among any of the 60 *T. gaigeae* mtDNA sequences generated for this study. Genetic distance values drawn from the control regions sequences for the groups are useful for comparisons among species and between populations of *T. gaigeae* (Table 3). Despite the lack of intrapopulational resolution, several individuals identified as *T. elegans* by phenotype were found to have the mtDNA of *T. gaigeae*. This is a clear indication of hybridization between the two taxa and was more frequent at the downstream end of the range. Interestingly, there were no individuals with the phenotype of *T. gaigeae*

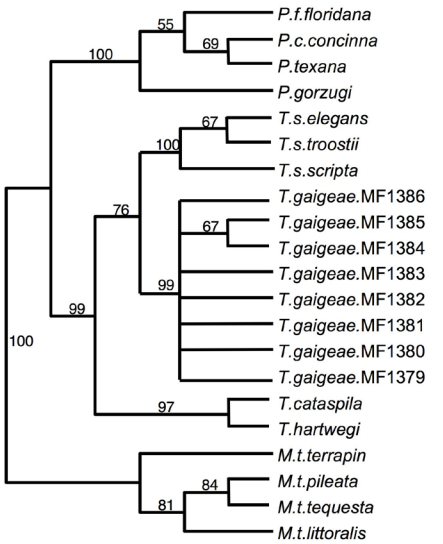

Fig. 3—Topology of phylogenetic relationships for selected several species in the Emydid general *Pseudemys* (P), *Trachemys* (T), and *Malaclemys* (M) resulting from 2500 parsimony bootstrap analyses (below the lines) of the 869 base pair mtDNA ND4-tRNA^{Leu} fragment. Neighbor Joining bootstrap analysis (above the lines) utilized the Kimura 2 parameter distance matrix correction.

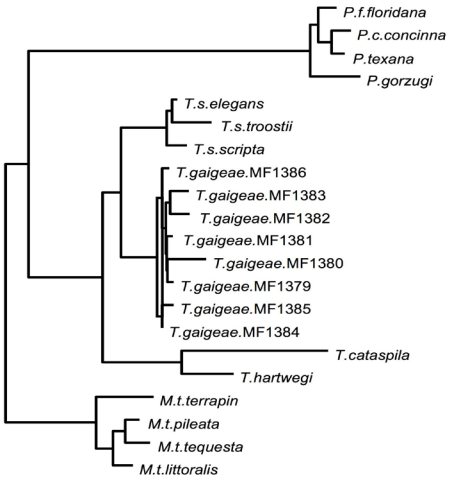

Fig. 4—Neighbor joining phylogram using the Kimura 2 parameter distance correction for the 969 base pair mtDNA ND4-tRNA^{Leu} fragment, containing 246 3' codons of NADH4, tRNA^{His}, tRNAser, and the majority of tRNA^{Leu} genes. In this figure, branch lengths are proportional to the number of changes on the topology.

and the mtDNA sequence of *T. elegans*.

Nuclear DNA microsatellite analyses—A total of 32 microsatellite loci were screened for this project. Of those markers, six were found to be polymorphic within *T. gaigeae* or between that species and *T. elegans*. Overall *T. gaigeae* demonstrates lower allelic variation for all nuclear markers than does its sister taxon. As alleles and allele frequencies differ between the two taxa, it was possible to use the microsatellite data to identify animals as *T. gaigeae*, *T. elegans*, or hybrids. Putative hybrids were identified in both New Mexico and Texas *T. gaigeae* populations. The samples were divided for initial comparison by separating the two species and subdividing the samples geographically. Table 4 provides a summary of the allelic variation for the polymorphic loci.

While Table 4 allows a rapid evaluation of the character of nuclear variation across all samples, the individual genotypes were also used to generate Gst and Rst matrices for all groups. In order to examine the structure within and between Texas and New Mexico, several geographically distinct groups were constructed. Each of these groups has been named and an acronym constructed based upon the origin of the largest number of samples within each group. The *T. elegans* samples were divided into *a priori* subgroups, predominantly taken from (1) the Rio Grande Nature Center in Albuquerque (NME, $n = 12$) where sliders are not native and the turtles are likely to have a wide range of origin; (2) a small number of individuals collected at the western edge of the native range of *T. elegans* in Texas (WTE, $n = 7$); (3) a central Texas group from the Nueces River (CTE, $n = 11$); and (4) a large sample of individuals from the Rio Grande drainage in extreme south Texas (STE, $n = 40$). The *T. gaigeae* were also partitioned by state and locality. Samples of *T. gaigeae* collected in Elephant Butte Reservoir, New Mexico were grouped (EBG, $n = 40$); as were animals taken from Bosque del Apache, New Mexico (BdAG, $n = 13$). In Texas the isolated population in Hudspeth County (IG, $n = 6$); is geographically disjunct from sample sites at Big Bend

TABLE 3—Genetic distance matrix calculated using the Tamura and Nei (1993) correction parameter for the mitochondrial control region sequences of several U.S. Emydid turtle genera and species.

	Taxon	1	2	3	4	5	6	7	8	9	10	11
1	*M. t. terrapin*	-										
2	*M. t. rhizophorarum*	0.05	0.01									
3	*Graptemys nigrinoda*	0.05	0.6	-								
4	*P. hieroglyphica*	0.06	0.07	0.07	-							
5	*P. c. concinna*	0.06	0.07	0.07	0	-						
6	*P. c. texana*	0.06	0.07	0.07	0	0	-					
7	*P. gorzugi*	0.06	0.07	0.07	0.01	0.16	0.02	-				
8	*T. s. elegans*	0.14	0.16	0.15	0.16	0.12	0.16	0.16	0.04			
9	South Texas *T. s. elegans*	0.09	0.10	0.11	0.11	0.10	0.10	0.12	0.04	0.04		
10	New Mexican *T. gaigeae*	0.08	0.10	0.09	0.09	0.10	0.10	0.10	0.04	0.04	-	
11	Texas *T. gaigeae*	0.08	0.10	0.09	0.09	0.10	0.10	0.10	0.04	0.04	0	-

TABLE 4—Summary of the microsatellite loci by locus, species, population and/or state. The values for each location represent the number of total alleles for that species in that locality and then parenthetically the alleles specific tot hat species and the alleles specific to that individual population.

Taxon	Locus	Total alleles	New Mexico* 12 *T.s.elegans* 52 *T gaigeae*	TX Rio N* 7 *T. s. elegans* 150 *T. gaigeae*	TX Rio S* 40 *T. s. elegans*	Nueces River* 11 *T. s. elegans*	Caribbean* 7 *T. s. elegans*
T. s. elegans	Galap3	24	15(12/6)	9(3/0)	15(8/1)	6(4/0)	8(5/0)
T. gaigeae	Galap3	8	7(0/2)	6(0/0)	n/a	n/a	n/a
T. s. elegans	Galap4	2	1(0/0)	1(0/0)	1(0/0)	2(1/1)	1(0)
T. gaigeae	Galap4	1	1(0)	1(0)	n/a	n/a	n/a
T. s. elegans	Pseud4-128	25	11(11/2)	5(3/0)	20(17/)	6(5/0)	10(9/1)
T. gaigeae	Pseud4-128	3	3(0/0)	3(0/0)	n/a	n/a	n/a
T. s. elegans	Pseud225-1	2	2(1/0)	1(0/0)	1(0/0)	2(1/0)	2(1/0)
T. gaigeae	Pseud225-1	1	1(0/0)	1(0/0)	n/a	n/a	n/a
T. s. elegans	Pseud225-2	5	4(0/0)	3(0/0)	4(0/0)	2(0/0)	2(0/0)
T. gaigeae	Pseud225-2	5	4(0/0)	3(0/0)	n/a	n/a	n/a
T. s. elegans	Tufu-2	14	9(1/0)	7(0/0)	14(4/2)	7(2/0)	5(2/0)
T. gaigeae	Tufu-2	13	9(1/0)	13(0/0)	n/a	n/a	n/a

*Key to the populations columns: New Mexico = all populations in New Mexico; TX Rio N = populations in the Rio Grande in Texas above Amistad Dam; TX Rio S = Rio Grande populations in Cameron County of extreme South Texas; Nueces River = samples taken in the Nueces River drainage of South Texas; Caribbean = samples of introduced *T. s. elegans* taken in Jamaica. Numbers represent the number of alleles (alleles specific to species/unique to specific population).

Ranch State Park (BBSRG, *n* = 43); western Big Bend National Park (WBBNPG, *n* = 18); eastern Big Bend National Park (EBBNPG, *n* = 44); and the Lower Canyons of the Rio Grande (LCG, n = 43). Finally, a group of *Trachemys decussata* (Tdecuss, *n* = 14) and now sympatric introduced *T. elegans* (JE, *n* = 7) collected in Jamaica were included in the analyses. Table 5 presents the Gst and Rst values calculated across all six loci by Microsat.C v1.5d (Minch 1997).

Subsequent analyses of the groups removed small individual populations (e.g. WTE, IG, and Jamaican samples) to examine differences derived from more robust population samples. No significant change in the distribution of population statistics occurred in that partitioning. Finally all samples of U.S. *T. elegans* (TSE) were grouped and all *T. gaigeae* samples were grouped by state of origin (New Mexico = NMTG and Texas = TXTG). The Rst values for TSE from TXTG is 0.413, from TSE to NMTG is 0.346, and between TXTG and NMTG is 0.009.

There are many unique alleles found in *T. s. elegans* (*n* = 42), but only one unique allele is found in *T. gaigeae* and this allele occurs only in New Mexico. Several alleles predominate in *T. s. elegans*, but are found in geographically concordant samples identified in the field as *T. gaigeae*. These individuals most likely represent animals of hybrid origin. These putative hybrids contain *T. gaigeae* mtDNA in every case and originate predominantly from Bosque del Apache in New Mexico and from at or below Bullis Fold in the Lower Canyons of the Rio Grande. Bullis Fold is a major geologic feature of the Lower Canyons at river kilometer 71 from the La Linda bridge on FM 2627 in Brewster County. While one *T. s. elegans* was collected much further upstream in Rio Grande Village (see above), the individual collected at Bullis Fold is more likely to mark the furthest natural upstream distribution of *T. s. elegans* in the Rio Grande. This site lies 56 river kilometers above the Brewster-Terrell county line and the individual in question demonstrated the normal head and shell markings of *T. s. elegans* except that there was no red within the dominant eyestripe. This particular individual possessed *T. gaigeae* mtDNA and its nuclear genotype included alleles from both *T. gaigeae* and *T. s. elegans*. The frequency of hybrid individuals is not dramatic within the Bosque del Apache (New Mexico) population (3/52 or 5%) nor the Texas populations (6/149 or 4%), however hybrids exist in more than just these two largest wild populations of *T. gaigeae*. We collected individuals demonstrating alleles found overwhelmingly in *T. s. elegans* at Rio Grande Village within eastern Big Bend National Park and within the isolated Hudspeth County population of *T. gaigeae*. The populations in western Big Bend National Park and those taken significantly upstream of Lajitas represent the only populations of *T. gaigeae* we sampled which did not contain any putative hybrid individuals.

TABLE 5—Genetic distance matrices calculated from six nuclear microsatellite loci. Matrices represent the summary of 298 individuals (14 *T. decussata*, 77 *T. s. elegans*, and 207 *T. gaigeae*) or 1,788 specific genotypes. See Results for key to the abbreviations used for each of the groups.

Gst Rst	NME	CTE	STE	WTE	LCG	EBBNPG	WBBNPG	BBSRG	IG	EBG	BdAG	Tdecuss	JE
NME	-	0.218	0.103	0.049	0.436	0.446	0.463	0.488	0.484	0.445	0.481	0.215	0.014
CTE	0.076	-	0.273	0.11	0.724	0.712	0.785	0.83	0.805	0.759	0.77	0.279	0.141
STE	0.369	0.187	-	0.065	0.439	0.444	0.465	0.502	0.496	0.445	0.47	0.313	0.142
WTE	0.279	0.133	0.001	-	0.251	0.261	0.278	0.307	0.325	0.29	0.292	0.217	0.027
LCG	0.629	0.388	0.527	0.638	-	0.003	0.008	0.014	0.038	0.04	0.035	0.651	0.538
EBBNPG	0.641	0.399	0.56	0.678	0.018	-	0.007	0.014	0.037	0.031	0.036	0.673	0.555
WBBNPG	0.578	0.312	0.616	0.568	0	0.027	-	0.008	0.024	0.023	0.018	0.726	0.598
BBSRG	0.665	0.41	0.578	0.696	0.002	0.017	0	-	0.039	0.033	0.046	0.766	0.623
IG	0.555	0.295	0.738	0.481	0.034	0.151	0.05	0.155	-	0	0	0.746	0.65
EBG	0.595	0.37	0.524	0.622	0.007	0	0.022	0.022	0.049	-	0.011	0.695	0.584
BdAG	0.516	0.277	0.612	0.484	0	0.041	0.015	0.05	0	0.001	-	0.715	0.63
Tdecuss	0.193	0.023	0.21	0.172	0.473	0.498	0.433	0.509	0.444	0.461	0.393	-	0.146
JE	0.251	0.044	0.203	0.136	0.755	0.773	0.67	0.783	0.589	0.741	0.61	0.104	-

Discussion—*Systematic status of* T. gaigeae—Many existing studies have drawn contradictory conclusions regarding the taxonomic and/or phylogenetic status of *T. gaigeae* (see above introduction). Recent studies of *Trachemys* systematics and taxonomy by Seidel et al. (1999) and Seidel (2002) using hemoglobin variability and morphometric analyses have helped clarify the relationship of *T. gaigeae* to its congeners and have provided support for recognition of this turtle as a distinct species. Our results based on DNA analysis are largely concordant with these earlier studies, excepting only the relationship between *T.hartwegi* and *T. gaigeae* proposed by Seidel (2002) and accepted by Stuart and Ernst (2004).

Based on the genetic information from both mitochondrial and nuclear DNA that was assembled for the current study, *T. gaigeae* is interpreted as a full species within the genus *Trachemys* (Fig. 3 and Table 3). Furthermore, its phylogenetic relationship within the genus based on DNA evidence does not conform to previously published conclusions (Iverson 1992; Smith and Smith 1979). The position of *T. gaigeae* is solidly supported by mtDNA data (Fig. 3) as the sister group to the North American *Trachemys* group. The DNA data do indicate a close relationship with the Middle American clade of *Trachemys* (Fig. 4). There is strong support (BP = 96%) for the distinction between two major groups of sliders (North American and Middle American) (Fig. 3). The separation of *T. scripta* (*sensu stricto*) from *T. gaigeae* is also conclusive (BP = 99%). On a larger scale, the Middle American *Trachemys* are the sister group of the northern *Trachemys* as has been previously hypothesized (Iverson 1992; Smith and Smith 1979; Seidel 2002), but the group does not include *T. gaigeae*. If similar comparisons are made using nuclear alleles from microsatellite loci (Table 3) then further support of this division is evident. Despite strong bias toward great numbers of *T. s. elegans* alleles at each locus (see below for further discussion) *T. gaigeae* maintains numerous unique species-specific alleles. Furthermore, the frequency distribution of alleles are also distinct when populations of *T. scripta* (sensu stricto) are compared to populations of *T. gaigeae*.

Behavioral differences between *T. gaigeae* and *T. s. elegans* also support the recognition of the former as a distinct species. Male courtship behavior in *T. gaigeae* (and some other Meso-South American *Trachemys*) involves rapid movements of the head ("head-nodding") and possible spraying of water from the nares towards the female, whereas males of North American sliders (*T. scripta, sensu stricto*) vibrate their elongated foreclaws against the face of the female (Seidel and Fritz 1997; Stuart and Miyashiro 1998). The presence of only *T. gaigeae* mtDNA in our samples of hybrid individuals suggests that only females of *T. gaigeae* are involved in hybrid events. One of us (JNS) observed

a successful copulation in captivity between a putative hybrid male with elongated foreclaws (which exhibited courtship behavior similar to that of *T. s. elegans*) and a *T. gaigeae*. It is possible that female *T. gaigeae* may be receptive to the courtship behavior of male *T. s. elegans*, but not the reverse, which would explain the occurrence of only *T. gaigeae* mtDNA in hybrids.

As *T. gaigeae* is genetically (this study), behaviorally (Stuart and Miyashiro 1998), and morphologically (Smith and Smith 1979; Seidel et al. 1999; Seidel 2002) distinct from *T. scripta* (*sensu stricto*), its recognition at the species level is justified. Recognition of *T. gaigeae* as a distinct species may also serve to focus needed conservation attention on this turtle and thereby lower the risk of extinction by inattention as has happened with other reptile species (e.g. unrecognized tuatara (*Sphendon* sp.; Daugherty et al. 1990).

Current Distribution—At present *T. gaigeae* is relatively abundant within what is presumably a vastly reduced distribution. While records of the taxon do not predate the construction of the New Mexico impoundments (Hartweg 1939), there is no reason not to believe that *T. gaigeae* was originally distributed from approximately Socorro, New Mexico downstream to the Brewster-Terrell county line in Texas. These two points represent the current north and south distributional boundaries for the range of *T. gaigeae* in the United States. No new localities for New Mexican populations (Degenhardt et al. 1996) were found in our survey. As a result of this survey, the range for *T. gaigeae* in Texas was tentatively extended to include Terrell County as the downstream limit of the species range (Dixon 2000). We base this conclusion on a very few specimens which have the phenotype and genotype of *T. gaigeae* and were trapped east of the confluence of Sanderson Canyon with the Rio Grande.

Habitat—As with many threatened taxa, *T. gaigeae* suffers primarily from human sponsored changes to its habitat. While it is apparent that *T. gaigeae* can and will adapt to a primarily lotic habitat (e.g. Elephant Butte Reservoir), in Texas it is found in association with heterogeneous riffle systems on the river. Figure 1 provides an overview of river flow rates from 1889 to 1975 gauged at several localities below El Paso. The figure provides a graphically accurate projection of the continual decrease in flow rates to the present, and also shows the impact of the impoundments in New Mexico (Fig. 1). Overall decreases occur just after each impoundment closed, but more threatening to the habitat is the trend of the regression plot (Fig. 1) demonstrating the decreased flow through time. Another way to examine this general trend is to examine the flow rates at individual gauging stations along the river (Fig. 5).

Figure 5 provides a graphical projection of the increase of river flow rates mitigated by the confluence with the Rio Conchos. The gauging stations above the confluence

show a very significant difference from stations at Alamito Creek or Langtry which both lie below the Rio Conchos confluence with the Rio Grande.

While it is apparent that the historical trends (Figs. 1 and 5) of economically damaging floods and intermittent flow rates have been successfully mitigated by the impoundments, these reservoirs have also radically affected most of the current range of *T. gaigeae* in the Rio Grande.

The dramatic inflow historically provided by the Rio Conchos to the Rio Grande is obvious (Fig 5). What is not apparent in that figure is the simple relationship between it and the current range of *T. gaigeae*. In effect the confluence of the Rio Conchos defines the upper boundary of the range of *T. gaigeae* in Texas. The exception to this is the isolated population which exists within the only remaining non-channelized portion of the river in Hudspeth County. Whether or not the Rio Conchos also provides a donor population base for *T. gaigeae* is impossible to determine from the present study.

The current distribution of *T. gaigeae* in the river is, largely, within either state or federally protected lands. This is a positive influence on the populations, but as demonstrated above, this will have little effect on the long term survival of the species in the wild without changes in the current water trends for the river itself. Furthermore, these turtles are not accorded any actual protection within those areas. It is completely

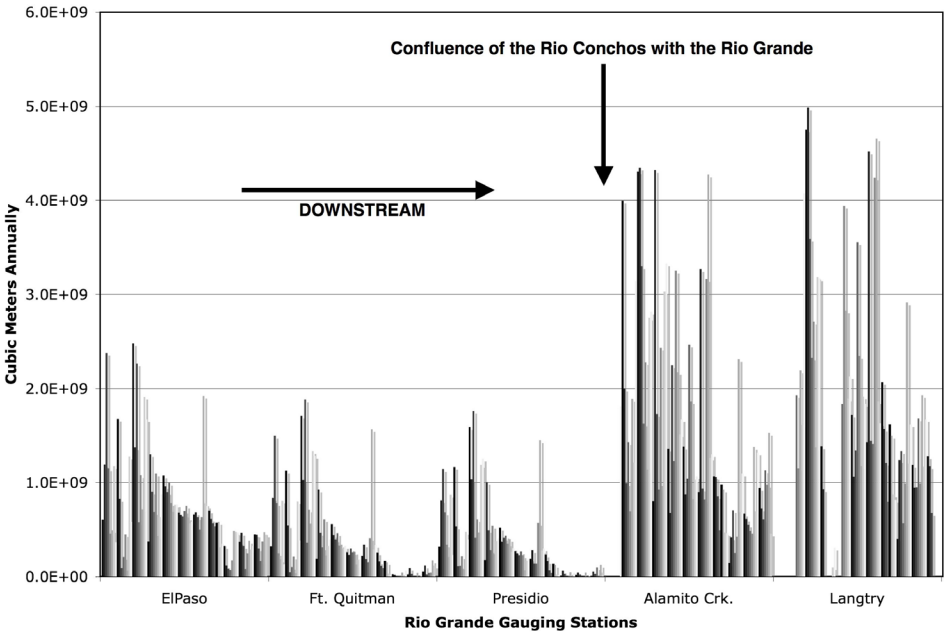

Figure 5— River flow rates at five gauging stations along the Rio Grande. Each station is recorded from 1889 to 1983 although gaps in individual records exist. The chart area for each station is annual flow plotted over that time period.

within the bounds of park and management area rules to take these turtles incidentally while fishing the river or impoundments. Within the boundaries of Big Bend Ranch State Park, Big Bend National Park, and Black Gap Wildlife Management Area this turtle is currently abundant. However, as noted for *P. gorzugi*, intensive removal efforts, such as by commercial reptile collectors, can pose a significant threat to turtle populations and can often go undetected. We were successful at every trapping location chosen within the current distribution of *T. gaigeae* (Fig. 2), an indication that populations in those areas are robust. However, we also recovered a high percentage (~20%) of individuals if we retrapped a given locality. This is an indication that the animals are sedentary, at least in part, along a particular portion of the river.

Hybrids—While we had previously detected hybridization at or near the Brewster-Terrell county line in Texas, we subsequently determined that *T. scripta elegans* x *T. gaigeae* hybrids are also found at Bosque del Apache National Wildlife Refuge in New Mexico, confirming the tentative conclusions of Seidel et al. (1999). These hybrids were identified in several ways, including both phenotypic and genetic markers. These individuals demonstrate both *T. gaigeae* mtDNA and nuclear microsatellite alleles which indicate a hybrid origin. In the southern end of the distribution for *T. gaigeae* we trapped several phenotypically distinct, and in some cases intermediate, turtles during our survey of that section of the river. These individuals possess *T. gaigeae* mtDNA and concurrently show varying degrees of microsatellite allele composition corroborating hybrid origin. Introduced *T. scripta* (presumably released pets) have been collected at Bosque del Apache (JNS, pers. obs.), thus the presence of hybrids here is not unexpected. Below the Big Bend of Texas, hybrids may result from the natural (parapatric) contact of native *T. s. elegans* with *T. gaigeae,* although introduction of non-native (pet trade) *T. s. elegans* at or above this contact zone may also be a contributor. The impact of this hybridization on *T. gaigeae* populations cannot be easily assessed at the present time.

Population structure—The results from the analyses of six polymorphic nuclear loci support the results of the mtDNA analyses. There are no significant differences among U.S. populations of *T. gaigeae* regardless of locality. The very low Rst (mean = 0.009) and Gst (mean = 0.023) values for the entire range in comparison with *T. s. elegans* (Rst = .244, Gst = 0.096) indicates significantly less genetic variation and population structure exists within *T. gaigeae* than within *T. s. elegans*. The population statistics may be artificially inflated for non-native *T. s. elegans* because these turtles are presumably derived from multiple locations. However, if the population of *T. s. elegans* from the Rio Grande downstream of Big Bend and the Nueces River in Texas (which likely represent native, non-introduced populations) are compared, the between *T. s. elegans* population

values (Rst = 0.187, Gst = .273) still greatly exceed the values between Texas and New Mexico *T. gaigeae* populations (see above). These results can be interpreted in several ways. The most likely cause of the lack of genetic variation in *T. gaigeae* across its range is its overall small range and consequently small population size in comparison with *T. s. elegans*. Also, the relatively recent (~100 years) separation of the New Mexico and Texas populations has not provided sufficient time for differences between the two groups to accumulate. It is also possible that the individuals in New Mexico represent introduced *T. gaigeae* populations, derived from the Rio Grande in Texas. While we view this as extremely unlikely, it is an alternative explanation of the complete lack of genetic differentiation between these two currently isolated groups. It is much more likely that *T. gaigeae* populations were recently (prior to the major dam construction in southern New Mexico) a single contiguous population that is now fragmented by a long reach of degraded habitat.

Current Population Estimates—Estimating the actual size of a population of wild animals is an exercise fraught with difficulties. In the current situation some of these difficulties are mitigated because *T. gaigeae* habitat is linear and our survey covered a significant proportion of the habitat. New Mexican populations are the most problematic as the artificial impoundments (reservoirs) provide heterogeneous habitat along the shorelines. Although we did not trap every kilometer of the river, we did trap numerous locations that provided a complete range from no or very few *T. gaigeae* to an abundance of this species. Based on this experience, we believe that we were able to develop a detailed search image for both the turtles and for their "preferred" habitat. We re-sampled two localities and successfully recovered previously marked animals (recapture frequency ~20% of 35 total individuals at two localities) to examine dispersal over a short time frame (two weeks). This is evidence that the overall population size at those localities is very likely to be small. This does not constitute sufficient data for statistically robust estimates of population size, however, it does provide evidence that *T. gaigeae* follows the same general pattern as other *Trachemys*, remaining in a defined range barring significant environmental change (Gibbons 1990). Our trap line was distributed along an average of one-half kilometer of river. While our average trap success was low (4%), in areas of optimal habitat trap success was much higher (12%). Using our trap success we classify 40% of the river within the current actual distribution of *T. gaigeae* (Fig. 2) as preferred habitat.

From our trap success, average length of our trap lines, recapture rates, and total number of individuals captured, we estimate 20 to 30 individuals for 2 km of optimal habitat. The range of *T. gaigeae* is roughly 480 km of the Rio Grande in Texas. Using

our estimate, ~200 km of the Rio Grande provides *T. gaigeae*'s preferred habitat of closely spaced riffles and pools. Thus, for Texas we liberally estimate the population to be between 2,400 to 3,600 individuals in preferred habitat. There were also *T. gaigeae* in marginal habitats, and we conservatively estimate an additional 360 to 900 individuals distributed through 290 km of river. For Texas we estimate the total *T. gaigeae* population to be less than 4,500 individuals. A generous estimate of the New Mexican populations would put the current number of individuals at two thirds of that figure, or 3,000 individuals. Thus, within the U.S. based on these obviously generalized estimates there are approximately 7,500 *T. gaigeae*. The status of Mexican populations remains uncertain, but based on what information we do have, we expect no more than 2,500 animals to be likely to occur in the Rio Conchos after impoundment events and anthropogenic flow changes with increased agriculture since the 1990s.

Our rough calculation provides an estimate of 10,000 *Trachemys gaigeae* in the wild. This figure is reflective of a population that has lost over half of its historic range due to channelization, impoundments, and irrigation demands on its habitat. Each of these physical modifications, exacerbated by recent severe drought in the Southwest, has compounded the problems wrought by introduced plant and vertebrate species to the Rio Grande. The population has been fragmented and geographically reduced by, at minimum, 50% since 1930. It is further compromised in both U.S. populations by introductions of *Trachemys scripta elegans*. As a final threat, we recently (2003 and 2004 surveys) have found the red imported fire ant (*Solenopsis invicta*) west of Amistad Reservoir and east of El Paso along the Rio Grande. As this pest moves west up the Rio Grande it will inevitably impact nests and hatchlings of *T. gaigeae* in the near future. Taken as a whole *Trachemys gaigeae* certainly meets IUCN criteria for "Vulnerable" status. Should any further habitat degradation occur, such as further inflow reductions to the Rio Grande or from the Rio Conchos, the taxon would likely decline. This in turn would necessitate immediate consideration as "Endangered" by IUCN criteria.

Recommendations for management—Management of the current populations of *T. gaigeae* may turn out to be problematical. As *T. gaigeae* populations in Texas are seemingly now dependent upon the mitigating influence of the Rio Conchos inflow (Fig. 5), that water source needs to be maintained. This is within the broader management problems of the Rio Grande drainage itself (Miyamoto et al. 1997). The river has been badly compromised for nearly the entire historical range of this turtle. Dredging and channelization of the river appears to have negatively impacted the distribution of *T. gaigeae* as it has wildlife in other river systems (Allen and Hardy 1980). Finally there are difficulties which are presented by the introduction of exotic pest species

to the drainage. While salt cedar (*Tamarix*) appears to be an excellent indicator of channelization and reduced flow, it is also never found in abundance with *T. gaigeae*. As salt cedar thickets tend to become nearly monocultural along the river banks (such is the case in western Presidio County above the confluence with the Rio Conchos) it is likely that turtles are unable to locate food. The consequences of the decline in native giant grasses (*Phragmites*) as the introduced (*Arundo donax*) species continues to expand is less clear. While it appears that *T. gaigeae* prefer *Phragmites*, it is plausible that they will eat *Arundo* shoots if no other choice is available.

The impact of introduced *T. s. elegans* that directly compete in the same habitat is less uncertain. Not only may *T. s. elegans* outcompete *T. gaigeae*, but it is apparent that *T. s. elegans* can and do hybridize with *T. gaigeae* populations when they are in sympatry. It is presently impossible to determine the future outcome of this process, but historical evidence from other species provides a grim picture (Avise and Nelson 1989; O'Brien et al. 1990; Lehman et al. 1991; O'Brien and Mayr 1991; Wayne and Jenks 1991; Dowling and Childs 1992; Roy et al. 1994).

The main portion of the current range of *T. gaigeae* lies within protected lands or lands held in the public trust. While this fact does influence our conclusions for management, it does not mean that *T. gaigeae* are safe within those boundaries. Fishing is a popular and allowed sport within these areas and turtles are routinely taken on hook and line, despite our dietary survey results. This is not likely to be a significant population drain but should be further examined to factually evaluate such an assumption. Furthermore, we have physical evidence that "pet" turtles (*T. s. elegans*) are released by well meaning, but ignorant, owners and subsequently survive in the wild within the boundaries of those protected areas. Continued genetic invasion of *T. gaigeae* populations by *T. s. elegans* is likely to represent as serious a threat to this species as any other.

The only protection currently afforded to *T. gaigeae* is a ban on commercial collection within New Mexico and the prohibition against collection on the federal lands where it occurs. One course of action might be to propose federal listing of *T. gaigeae* as an endangered species. It has many of the characteristics of an endangered species (e.g., small total distribution, compromised habitat, continued habitat destruction likely, etc.). However, we do not believe that listing of *T. gaigeae* would be necessarily in the best interest of this taxon. If the taxon were listed it might become more desirable to commercial collectors. Enforcement of collecting bans in the isolated and largely unpopulated range of *T. gaigeae* is nearly impossible. We later learned that this was accomplished by a single group of one or a few commercial collectors. We fear a similar

outcome for *T. gaigeae* should "official" notification of its status be made, although the relative inaccessibility and land-ownership of many sites occupied by the species may afford some protection from over-collection.

Conservation of *T. gaigeae* is achievable through two primary approaches: management of the Rio Grande to protect and enhance the native ecosystem, and the elimination of non-native *T. s. elegans* occurrence in the range. Just as in the Colorado River (Stevens et al. 1995), the historic seasonal flood patterns may need to be reestablished in the Rio Grande to support the natural biological processes on which the river's fauna and flora depend. While we were unable to locate information from the Mexican government that detailed any plans for further impoundment of the Rio Conchos, it is a near certainty that such will eventually be proposed and executed. While we were examining *T. gaigeae* we also observed many other wildlife species which share similar modified ranges (softshell turtles, gar, native mollusks) and suffer negative effects of the current quality of the ecosystem. Preservation of *T. gaigeae* populations by management of the drainage itself would benefit a host of other species. Managers and management authorities should pay special attention to the above section regarding the status and numbers of *T. gaigeae* in the wild. Should the Rio Grande be any further compromised, the necessity and urgency of IUCN Endangered status listing for the taxon will be imperative.

The now widely introduced red-eared slider (*T. s. elegans*) represents one aspect of the threat to *T. gaigeae* which can be mitigated. While it is impossible to isolate or remove all hybrids within the populations, efforts can be made to remove *T. s. elegans* from these populations whenever encountered by qualified personnel. While hybrids themselves are not always phenotypically apparent, *T. s. elegans* phenotypes are quite distinctive. Removal programs are unlikely to be as successful as an active program which seeks to prevent additional releases into the river. Anecdotal evidence from our interviews during the 1998 surveys and since then, suggests that at least some of the introductions made within the boundaries of Big Bend National Park were done by persons unaware that their released pets posed a threat to native slider populations. If public awareness of the disastrous effects of such introductions were increased in the local area schools and towns, it is likely that such introductions would diminish rapidly. One of us has recently completed a project in collaboration with the Ft. Worth Zoo and Texas Parks and Wildlife creating a poster on Texas turtles targeting this audience. Park personnel should remain aware of this problem and continue to address it in their visitor education programs and individual capacity whenever possible.

This study provides a major first step in understanding the status of *T. gaigeae*

throughout its U.S. range. There should be continued (smaller scale) efforts devoted to obtaining samples over time using the current study as the baseline information for the populations. Future studies can utilize the data collected here to determine population trends for *T. gaigeae*. Further examination of the Rio Grande from the Terrell County line to the confluence with the Pecos River is also warranted. While the current study attempted to sample this region, results were negligible. An effort to ascertain the eastern limits of *T. gaigeae* alleles and of the western edge for "pure" *T. s. elegans* should be made. Furthermore, the examination of the density of turtles in that section of the river is also important. Knowing how many *T. s. elegans* are reaching the eastern boundary of *T. gaigeae*'s range will further assist with the evaluation of the effect of *T. s. elegans* intrusion into the range of *T. gaigeae*. Historical records (Texas Cooperative Wildlife Collection) fail to provide any evidence of *T. s. elegans* in the Rio Grande west of Val Verde County (near the Pecos confluence) until 1994; this suggests either inadequate sampling prior to the mid-1990s or possible changes in the river ecosystem that encourage the upriver dispersal of *T. s. elegans*. Finally, the current distribution, abundance, and genetic character of Mexican populations of *T. gaigeae* in the Rio Conchos drainage system should be evaluated in coordination and collaboration with Mexican scientists and authorities.

Conclusions—The preliminary genetic evidence assembled for this project support *T. gaigeae* as a full species and a unique taxon among U.S. turtle species. That preliminary evidence is now supported by extensive sampling both physically across the turtle's distribution and genetically from multiple DNA markers. While *T. gaigeae* had been known to science for a relatively long period of time it had not been examined as a species across its range and little was known about current population status. Its range lies in a spectacularly wild and unpopulated section of the U.S. Counterintuitively, the river it depends on is threatened by rapidly growing human populations and concurrent demands for water upstream. The needs of those human populations have resulted in the loss of at least half of *T. gaigeae's* total range in the U.S. and split the remaining populations into two geographically separate ranges.

While for many years *T. gaigeae* has been able to persist in relative obscurity, the growing human demands on the Rio Grande, especially during periods of extreme drought, have resulted in greater threats to the river's wildlife. Creation of a sound water management strategy for the Rio Grande drainage should minimize the impact of existing and future conflicts. The current study provides information we believe will be useful to resource managers in the river basin. This turtle's current precarious position and distribution is paralleled by other declining organisms (e.g., the federally

endangered Rio Grande silvery minnow and southwestern willow flycatcher) that have been impacted by anthropogenic changes to the Rio Grande drainage. The most positive aspect of the current status of *T. gaigeae* in the wild is that we are now aware of the human impact on these populations and can take steps to mitigate the damage already done.

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