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The Status of *Apalone atra* Populations in Cuatro Ciénegas, Coahuila, México: Preliminary Data

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ABSTRACT. – **The species-level designation of the Mexican softshell turtle, *Apalone atra*, has been repeatedly challenged, yet no DNA evidence has been collected. We conducted field studies of all the drainages of the Cuatro Ciénegas basin, and the only pure morphological population of *A. atra* found was in Tío Candido, the type locality for the species. One nuclear intron known to show species-level divergence in the family Trionychidae, 2 nuclear genes, and a mitochondrial gene revealed little molecular divergence for *A. atra* when compared with *A. spinifera emoryi* from the Rio Grande. Further, no reciprocal monophyly of the mitochondrial gene tree was seen between *A. atra* and *A. s. emoryi* morphotypes.**

For many species, hybridization and high migration rates are important components of an organism's long-term evolutionary trajectory (Maddison 1997), but in a short time scale, these behaviors create difficulty in the delimitation of these organisms as evolutionarily significant units (ESU) for conservation protection (Moritz 1994). ESUs are defined in 2 ways: 1) populations that have reciprocal monophyly at mitochondrial loci with significant divergence in nuclear loci (Moritz 1994) and 2) populations that are substantially reproductively isolated and represent distinct evolutionary legacies (i.e., genetic variability; Waples 1991, 1995). Here we investigate DNA evidence to determine if the turtle species *Apalone atra*, the endemic Cuatro Ciénegas black softshell, meets either of these criteria.

The taxonomic and conservation standing of *A. atra* is controversial. This turtle is currently listed on the CITES (Convention on International Trade in Endangered Species of Wild Flora and Fauna) Appendix I endangered species list, but it has also been reported to be extinct (Smith and Smith 1979). Hybridization between *A. atra* and a south Texas species, *Apalone spinifera emoryi*, is thought to have begun in the 1880s when irrigation canals were constructed, opening the Cuatro Ciénegas basin hydrologically (D. Hendrickson, *pers. comm.*; Webb 1973). Winokur (1968) could not determine the rate of hybridization, concluded the identification of some specimens as hybrids to be uncertain, and verified pure *A. atra*. By 1979, Smith and Smith considered *A. atra* to be an extinct lineage due to hybridization with *A. spinifera emoryi.* In 1983, a softshell resembling pure *A. atra* was noted in a field trip to the Cuatro Ciénegas basin (Ernst and Barbour 1992).

In this study, we sought to illuminate the taxonomic status of the endemic Cuatro Ciénegas black softshell, *A. atra*, by assessing its molecular distinction from the invasive congener, *A. s. emoryi*. We sampled all 5 drainages in the basin (Evans 2005) for turtles with *A. atra* morphological characteristics and used mitochondrial DNA sequence data from Cuatro Ciénegas softshell turtles and *A. spinifera* subspecies to reconstruct a haplotype network and a phylogenetic tree. In addition, we built distance matrices with the mitochondrial DNA data and 3 nuclear loci.

Methods. — Morphological surveys were extensive; we inspected all currently defined and flowing drainages in El Área de Protección de Flora y Fauna Cuatro Ciénegas, Coahuila, México, for *A. atra* (Fig. 1; Minckley 1969). This sampling included the type locality, Tío Candido, and spanned 62 days of trapping from 16 May to 16 June 2003 and from 4 June to 5 July 2004 (Webb and Legler 1960). Turtles were captured in lobster or hoop traps that were baited with sardines and checked every 12–14 hours. Each turtle was tattooed in a unique pattern on the plastron.

Morphologically, *A. atra* is characterized by 5 main traits: 1) "blackish" pigmentation of dorsal surface, 2) speckled pigmentation on ventral surfaces, 3) faint marginal bands and posterior white tubercles on the carapace of males only, 4) longitudinal corrugations on the posterior of the carapace, and 5) ovoid adult carapaces (Webb and Legler 1960; Winokur 1968). *Apalone spinifera emoryi* is defined by 1) tan to olive-brown carapace; 2) no ventral pigmentation; 3) defined pale marginal band; 4) white, raised tubercles on the back third of the carapace; and 4) a clear triangular facial pattern (Ernst et al. 1994). In this study, *A. atra* were distinguished from *A. s. emoryi* by dark pigmentation on the carapace, speckled pigmentation on the plastron, and corrugations on the posterior carapace. These traits showed no sexual dimorphism and little to no change through adult life and were exclusive to *A. atra*. See Appendix I for morphological characteristics and habitat types and Appendix II for photo voucher accession numbers for each specimen.

Less than 0.5 ml of blood were drawn from the caudal vein of the 26 field-collected animals, stored in buffer (0.01 M Tris, 10 mM EDTA, 0.01 M NaCl, and 1% SDS), and frozen. DNA was extracted using Roche High Pure Template Preparation Kit (Cat. 1796828). DNA sequence data were obtained using an ABI 3730 DNA Analyzer.

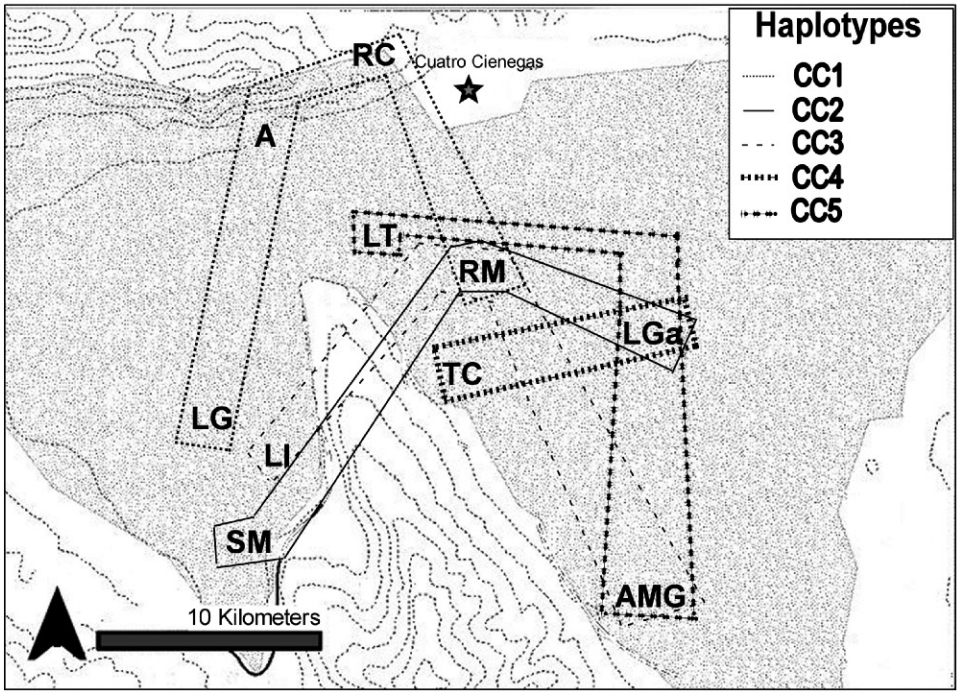

Figure 1. Approximate localities sampled in the Cuatro Ciénegas basin. Map adapted from Moline et al. (2004). Populations include AM: Anteojo; AMG: Antiguos Mineros Grande; LG: Laguna Grande; LGa: Los Gatos; LI: Laguna Intermedia; LT: Posa de las Tortugas; TC: Tio Candido; RC: Rio Cañón; RM: Rio Mesquites at Las Salinas; SM: San Marcos. Laguna Intermedia and Laguna Grande are connected by a short canal and considered 1 site. Rio Cañón is located north of the town of Cuatro Ciénegas. Morphological but not molecular data were taken from drainage 4.

This included 725 base pairs (bp) of cytochrome *b* mitochondrial gene (CytbF: 5′ ACAGGCGTAATCC-TACTAC 3′; DW1594; see Weisrock and Janzen 2000) from putative *A. atra* in the type locality ($n = 6$), 8 other localities in the basin ($n = 19$), and the pallid softshell (*A. s. pallida*; MidCon63). Additionally, 1638 bp of recombination activating gene-1 (RAG-1; see Krenz et al. 2005 for primers), 886 bp of RNA fingerprint protein 35 nuclear intron (R35; see Fujita et al. 2004 for primers), and 538 bp of the oncogene C-*mos* (see Saint et al. 1998 for primers) were used to construct distance matrices to compared the type locality specimens ($n = 2$) to *A. s. pallida* (CME63 from Irion County, Texas), *A. s. emoryi* (TXsc from Valverde County, Texas, and NMrg from Socorro County, New Mexico; Weisrock and Janzen 2000), and *A. mutica* (LAcrm1 from East Baton Rouge Parish, Baker, Louisiana; Weisrock and Janzen 2000). The nuclear intron R35 has been successful in resolving species-level phylogenies in the family Trionychidae (Engstrom et al. 2004), and RAG-1 has been successful in resolving species-level phylogenies in other Testudines (Krenz et al. 2005). The third nuclear marker, C-*mos*, has not been extensively used for phylogenetics in turtles. All PCR products were gel purified for the appropriate band size using Qiagen's QIAquick Gel Extraction Kit (Cat. 28706). Some heterozygosity was observed in the RAG-1 sequences. As needed, we cloned separate alleles using pGEM-T Easy Vector System I (Promega A1360) and One Shot Mach I Competent Cells (Invitrogen C8620–03). GenBank accession numbers are located in Appendix II.

Eight additional samples from a previous study were used for the total phylogenetic analysis (total $n = 34$; Weisrock and Janzen 2000; LAcr1m, NMrg, ONtr1, FLer1, GAsr, TXcc, TXki, and TXsc). Alignments were performed in CLUSTAL W (Thompson et al. 1994), and sequences were visually reviewed and corrected in BioEdit 7.0.0 (Hall 1999). Modeltest 3.7 (Posada and Crandall 1998) was used to estimate parameters of sequence evolution for all genes using Akaike's information criterion (Posada and Buckley 2004). No model of sequence evolution was proposed or tested in other studies of *Apalone* phylogeography (e.g., Weisrock and Janzen 2000), but the models for cytochrome *b* in this study were tested with representatives from major clades of Weisrock and Janzen (2000). These parameters were taken into account when constructing distance matrices in PHYLIP 3.62 (Felsenstein 2005). A maximum likelihood tree using cytochrome *b* was constructed using parameters estimated in Modeltest 3.7 and bootstrapped for 100 replicates using Paup 4.0b. Parsimony and neighbor-joining trees carried the same signature of weakly supported nodes and can be obtained from the first author. Nuclear DNA sequences showed little to no divergence, and therefore, no trees were constructed using those data. Haplotype groups were defined with DNASP (Rozas et al. 2003). Haplotype networks were built using statistical parsimony in TCS with gaps set as missing data (Clement et al. 2000).

Results. — Animals that were morphologically concordant with *A. atra* were found predominantly in the type locality, Tío Candido (Fig. 2). Here, no *A. s. emoryi*

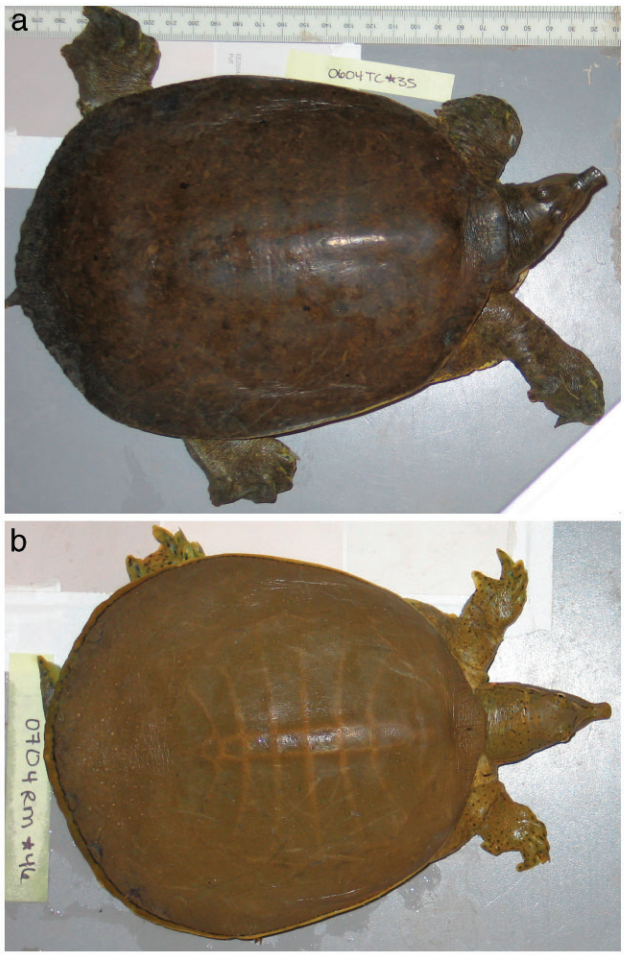

Figure 2. Putative *Apalone atra* from Tio Candido (TC35, female, 2a) and *Apalone spinifera emoryi* from the Rio Mesquites at Las Salinas (RM25, male, 2b). *Apalone atra* is characterized by dark, "blackish" pigmentation of dorsal and ventral surfaces and longitudinal corrugations on the posterior part of the carapace. Defining characteristics for *A. s. emoryi* are a tan or olive-brown carapace with the back third covered in white tubercles. Photos by S. McGaugh.

morphs were found in 11 days of trapping (Webb and Legler 1960). Turtles trapped in Antiguos Mineros Grande, and 3 turtles trapped in Posa de las Tortugas (e.g., LT104; also known as Mojarral Este) also were morphologically concordant to the description of *A. atra*, but *A. s. emoryi* morphs and potential hybrids were also present. Within the other 3 sites, Antejeo, Rio Mesquites, and Rio Cañón, *A. atra*–like traits were found in conjunction with *A. s.* emoryi–like traits in the same animal. Hybrids and backcrosses were difficult to accurately define; although, 9 turtles presented both *A. s. emoryi* and *A. atra* characteristics (Appendix I). Only morphological characteristics of *A. s. emoryi* were found at San Marcos, Los Gatos, and Laguna Grande.

Results from distance matrices are illustrated in Table 1. Cytochrome *b* divergence of *A. atra* (TC38 and TC36, Table 1) with unequivocal species and subspecies, *A. mutica* (~ 9.24%) and *A. s. pallida* (1.24%), was substantial. Cytochrome *b* divergence of *A. atra* with *A. s. emoryi* was nearly an order of magnitude lower (0.14%–0.28%). Likewise, the nuclear loci showed divergence between *A. mutica* and *A. atra* (RAG-1: 0.93%, R35: 1.14%, C-*mos*: 0.58%) but very little divergence between *A. s. emoryi* and *A. atra* (RAG-1 < 0.01%, R35: 0.10%, C-mos: 0.0.09%). *Apalone atra* did not show substantial divergence with *A. s. pallida* at these nuclear loci either (RAG-1 < 0.01%, R35 < 0.01%, C-mos: 0.14). This combined genetic information, with special emphasis on the cytochrome *b* data, suggests that sequence divergence between the Rio Grande *A. s. emoryi* (Txsc and NMrg; Weisrock and Janzen 2000) and individuals from the type locality for *A. atra*, Tío Candido, where *A. s. emoryi* morphs were never caught, is insufficient for strong ESU delimitation through Waples's (1991, 1995) requirement of reproductive isolation.

Results from the phylogenetic analysis strengthened the hypothesis that *A. atra* does not represent a unique species. The maximum likelihood tree is illustrative of all trees constructed (Fig. 3). The lack of reciprocal monophyly of *A. atra* morphs in comparison to *A. s. emoryi* morphs suggests that *A. atra* does not qualify as an ESU through Mortiz's (1994) definition (Fig. 3).

Nine mitochondrial haplotypes were recorded among 25 individuals from the 9 sites in the Cuatro Ciénegas basin, 2 Rio Grande *A. s. emoryi* samples (TXsc and

Table 1. DNA pairwise distances of *Apalone spinifera emoryi* (TXsc and NMrg), *A. atra* (Tío Candido, Cuatro Ciénegas, México; TC38 and TC36), *A. s. pallida* (Irion County, TX; CME63), and *A. mutica*. Values are given in percentages for 725 base pairs (bp) of cytochrome *b*, 1638 bp of *recombination activase gene-1*, 886 bp of RNA fingerprint protein 35 nuclear intron, and 538 bp of C-*mos*, respectively.

	A. atra (TC36)	*A. atra* (TC38)	*A. s. emoryi* (TXsc)	*A. s. emoryi* (NMrg)	*A. s. pallida* (CME63)
A. atra (TC36)	0				
A. atra (TC38)	0.001, 0.001, 0.001, 0.100				
A. s. emoryi (TXsc)	0.276, 0.001, 0.001, 0.092	0.276, 0.001, 0.001, 0.092			
A. s. emoryi (NMrg)	0.139, 0.001 0.103, 0.0911	0.139, 0.001 0.103, 0.091	0.139, 0.001, 0.103, 0.061		
A. s. pallida (CME63)	1.261, 0.001, 0.001, 0.141	1.261, 0.001, 0.001, 0.141	1.261, 0.001, 0.001, 0.071	1.120, 0.001, 0.103, 0.071	
A. mutica	9.243, 0.934, 1.144, 0.582	9.243, 0.939, 1.144, 0.582	9.249, 0.939, 1.144, 0.550	9.085, 0.939, 1.2492, 0.551	8.447, 0.939, 1.144, 0.176

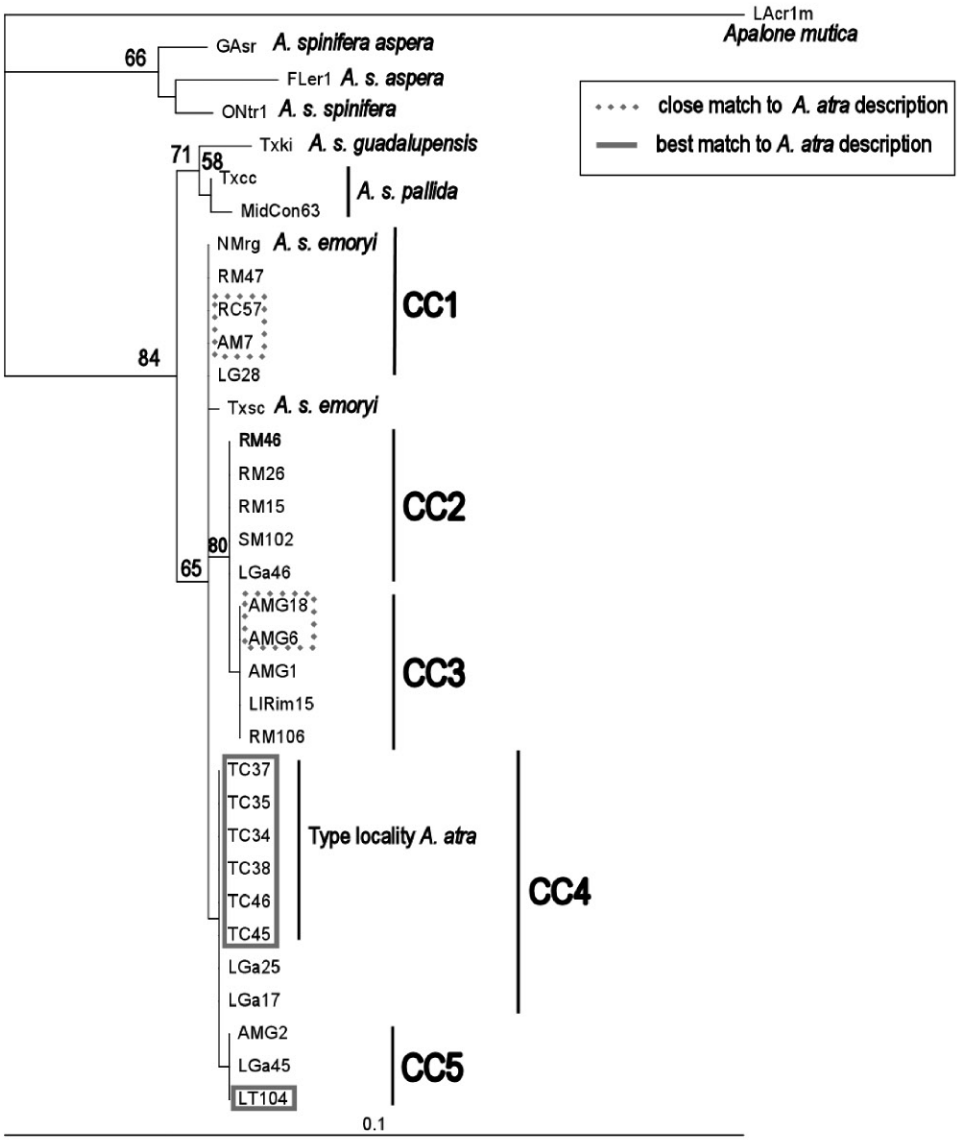

Figure 3. Maximum likelihood tree for cytochrome *b* samples within the Cuatro Ciénegas basin. Bootstrap values are given. *Apalone spinifera* subspecies are given in the tree, including *A. s. emoryi* from the Rio Grande (NMrg: Socorro County, New Mexico; TXsc: Valverde County, Texas; see Appendix I for full descriptions). The out-group is *A. mutica* (Lacr1m) from East Baton Rouge Parish, Baker, Louisiana. Haplotypes are defined only within the Cuatro Ciénegas basin. Solid gray boxes indicate those specimens that were most concordant with species descriptions of *A. atra*. Dotted gray boxes indicate specimens that are very similar but not perfectly concordant to species descriptions of *A. atra*.

NMrg), 2 south Texas *A. s. pallida* samples (TXcc and MidCon63), and 1 southeast Texas *A. s. guadalupensis* sample (TXki; Fig. 4). Although for a within-population analysis including south Texas *Apalone* may not be accurate, including these samples provided perspective on the close relationship of *A. s. emoryi* and *A. atra* haplotypes. Five of the 9 mitochondrial haplotypes (CC1–CC5) occurred in the Cuatro Ciénegas basin, with 1 of those 5 (CC1) containing a Rio Grande sample (NMrg; Fig. 4). One mitochondrial haplotype (CC4) was shared among all Tío Candido turtles, and 2 other turtles from another eastern locality in the basin (Los Gatos; Fig. 4). This preliminary sampling was not sufficient to perform statistical evaluations of haplotype genealogy and diversity, but examination of the geographic distribution of haplotypes (Fig. 1) suggests that some microgeographic genetic structuring may potentially exist.

Discussion. — Weisrock and Janzen (2000) reported that *A. spinifera* and *A. mutica* exhibit relatively large amounts of intraspecific mitochondrial cytochrome *b* DNA variation. This marker is sufficiently variable to reveal sequence divergences between legitimate species (e.g., *A. spinifera* and *A. mutica* are 8.0%–8.8% divergent from one another; Weisrock and Janzen 2000). Two Rio Grande populations of *A. s. emoryi*, Socorro County, New Mexico, and Valverde County, Texas, have low sequence divergence for cytochrome *b* (0.1%), and these samples served as primary references for evaluating the genetic divergence between *A. atra* and *A. s. emoryi* morphotypes in the Cuatro Ciénegas basin. We found that sequence diver-

gences between the Rio Grande *A. s. emoryi* and the type locality for *A. atra* in the Tio Candido drainage of Cuatro Ciénegas (0.14%–0.28%) are comparable to the within–Rio Grande levels of divergence for *A. s. emoryi*, providing no mitochondrial evidence of previous speciation between these taxa. In the case of sexually symmetrical hybridization, one would expect to find a distinct, remnant mitochondrial signature if the populations were separate in the recent (~ 130 years) past. No evidence for asymmetrical gene flow (from *A. s. emoryi* females to *A. atra* males) is apparent, and so the homogeneous nature of haplotypes reported here supports the idea that these putative species were probably not historically separate in gene flow.

The phylogenetic and haplotype analyses of the mitochondrial data indicate that there is nonreciprocal monophyly between morphologically putative *A. atra*, the *A. s. emoryi* morphotypes in the basin, or Rio Grande individuals (Figs. 3, 4). With such weak divergences and low bootstrap support for within-basin comparisons (Fig. 3), important insight can be drawn from a haplotype network (Fig. 4) in conjunction with the phylogenetic analysis. Network examination reveals greater diversity is present between haplotypes within the Cuatro Ciénegas basin than between the *A. s. emoryi* of the Rio Grande and *A. atra*. Although type locality individuals (Tío Candido) are all of the same haplotype (CC4), Los Gatos individuals are from the same haplotype group and are morphologically *A. s. emoryi*.

The haplotype analysis is probably unaffected by the analysis of only 2 samples of *A. s. emoryi*. These samples are separated by greater than 1100 km of river distance; still, the haplotypes were only 0.1% divergent from one another (Weisrock and Janzen 2000). Thus, it could be safely assumed that the total divergence across the entire range of *A. s. emoryi* is probably similarly low. Even with more extensive *A. s. emoryi* sampling, haplotypes will most likely continue to be very similar to those found in the Cuatro Ciénegas basin.

At least 2 factors must be considered before conservation management recommendations are made. First, morphological variation within the basin is abundant and could be indicative of real, locally adapted morphs. Winokur (1968) hypothesized that this morphological variability may be related to habitat type. Overall, darker, *A. atra*–like individuals were found in dark-bottomed lagoons, while lighter, *A. s. emoryi*–like individuals were found in light-bottomed playa lakes and rivers (Winokur 1968; Webb 1973). This view is supported, at least, for coloration, a trait that is hypothesized here to be a result of background matching (Appendix I; McGaugh 2008). Background matching is a physiological color change in response to light or dark surroundings and is known to happen over several weeks to months in *A. spinifera* (Bartley 1971; Ernst et al. 1994). Second, even if the morphological variability can be potentially explained by habitat parameters, the limited geographic distribution of

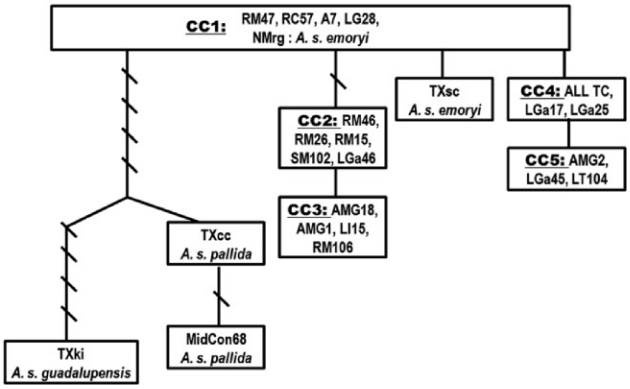

Figure 4. Haplotype network of cytochrome *b* generated by statistical parsimony. Each vertical bar represents 1 mutation, and diagonal bars separate mutation events. Haplotypes are named only within the Cuatro Ciénegas basin. It is clear from the network that *Apalone* from the Cuatro Ciénegas basin share or are very close to *A. s. emoryi* (NMrg and TXsc) haplotypes. More variability occurs within the Cuatro Ciénegas basin than between the 2 putative taxa (*A. atra* and *A. s. emoryi*). Haplotypes from other Texas *A. spinifera* subspecies (TXki: *A. s. guadalupensis*; TXcc and MidCon63: *A. s. pallida*) are more distant to each other and to the *A. s. emoryi*-Cuatro Ciénegas haplotypes than the *A. s. emoryi* and Cuatro Ciénegas haplotypes are to each other.

mitochondrial haplotype CC4 suggests that some genetic substructuring may potentially exist. An analysis of mitochondrial haplotypes containing more individuals and incorporating population-level nuclear markers, such as microsatellites or amplified fragment length polymorphism, could help delimit management units within the Cuatro Ciénegas basin (Moline et al. 2004; Carson and Dowling 2006).

Our analysis exemplifies a notable problem of current biology and taxonomy: delimiting species and ESUs. In the last thorough examination, Winokur (1968) maintained the status of *A. atra* as a distinct species based on the assumption that gene flow was prezygotically restricted between *A. atra* and *A. s. emoryi* by ecological preferences. Our analysis reiterates that morphological variation is associated with habitat type (*A. atra*–like animals in lagoons and *A. s. emoryi*–like individuals in rivers and playa lakes) but demonstrates little to no molecular distinctions and no reciprocal monophyly between the animals in the Cuatro Ciénegas basin and those in the Rio Grande. These data provide strong evidence that *Apalone atra* is not a separate species from *Apalone spinifera emoryi*.

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Appendix I. Sample names are given along with locality, habitat type, and morphological identification.

Sample	Locality and morphological information
LAcr1m*	Comite River, East Baton Rouge Parish Baker, Louisiana, *A. mutica*
TXsc*	Sycamore Creek, Valverde County, Texas, *A. s. emoryi*
NMrg*	North Elephant Butte Reservoir, Socorro County, New Mexico, *A. s. emoryi*
FLer1*	Escambia River, Escambia County, Florida, *A. s. aspera*
Gasr*	Suwanee River, Lanier County, Georgia, *A. s. aspera*
ONtr1*	Thames River, north of London, Ontario, Canada, *A. s. spinifera*
TXki*	Kingsville, Kleber County, Texas, *A. s. guadalupensis*
TXcc*	Coleto Creek, Goliad County, Texas, *A. s. pallida*
MidCon63	Middle Concho River, Irion County, Texas, *A. s. pallida*
TC34mt	Tio Candido, *A. atra* type locality: Lagoon, *Rugose margin, dark coloration on carapace and dark markings on plastron*
TC35mt	Tio Candido, *A. atra* type locality: Lagoon, *Rugose margin, dark coloration on carapace and dark markings on plastron*
TC37mt	Tio Candido, *A. atra* type locality: Lagoon, *Rugose margin, dark coloration on carapace and dark markings on plastron*
TC38mt	Tio Candido, *A. atra* type locality: Lagoon, *Rugose margin, dark coloration on carapace and dark markings on plastron*
TC36mt	Tio Candido, *A. atra* type locality: Lagoon, *Rugose margin, dark coloration on carapace and dark markings on plastron*
TC45mt	Tio Candido, *A. atra* type locality: Lagoon, *Rugose margin, dark coloration on carapace and dark markings on plastron*
AM7mt	Antejeo: Lagoon, *Slightly rugose margin, medium dark carapace pigmentation, few markings on plastron*
AMG18	Antiguos Mineros Grande: Lagoon, *Slightly rugose margin, dark pigmentation on carapace, some dark markings on plastron*
AMGst6	Antiguos Mineros Grande: Lagoon, *Slightly rugose margin, dark pigmentation on carapace, some dark markings on plastron*
AMGst1	Antiguos Mineros Grande: Lagoon, *Margin very slightly rugose, medium dark pigmentation on carapace, few dark markings on plastron*
AMGst2	Antiguos Mineros Grande: Lagoon, *Margins not rugose, medium dark pigmentation on carapace, no dark markings on plastron*
LT104	Morjarral Este: Lagoon, *Rugose margins, dark pigmentation, dark markings on plastron*
SM102	Eijido San Marcos: Waste water pond, *Margins not rugose, no dark pigmentation*
RM46mt	Rio Mesquites: River, *Margin not rugose, medium dark carapace pigmentation, no markings on plastron*
RM26mt	Rio Mesquites: River, *Slightly rugose margin, dark carapace pigmentation, few markings on plastron*
RM47mt	Rio Mesquites: River, *Slightly rugose margin, medium dark carapace pigmentation, few dark markings on plastron*
RM106	Rio Mesquites: River, *Slightly rugose margin, medium dark pigmentation, few dark markings on plastron*
RM15mt	Rio Mesquites: River, *Margin not rugose, medium dark pigmentation, no markings on plastron*
RC57mt	Rio Cañón: River, *Very slightly rugose margin, dark pigmentation on carapace, some dark markings on plastron*
LGst28	Laguna Grande: Playa lake, *Margin not rugose, no dark pigmentation*
LIRim15	Laguna Intermedia: Playa lake, *Slightly rugose margin, no dark pigmentation*
LGa46	Los Gatos: Playa lake, *Margins not rugose, no dark pigmentation*
LGa45	Los Gatos: Playa lake, *Margins not rugose, no dark pigmentation*
LGa25	Los Gatos: Playa lake, *Margins not rugose, no dark pigmentation*
LGaRIM17	Los Gatos: Playa lake, *Margins not rugose, no dark pigmentation*

Appendix II. GenBank and photo voucher accession numbers. An asterisk denotes sequences obtained from GenBank; all but R35 for LAcrm were obtained from Weisrock and Janzen (2000). Photo vouchers are not available for these GenBank specimens, and this is indicated as NA. GenBank accession numbers are given for cytochrome *b*, RAG-1, C-*mos*, and R35, respectively, and separated by semicolons for each gene. Alleles for each gene are separated by commas.

LAcr1m*	NA; AF168766*; DQ529173; DQ529206; AY259581.1*
FLer1*	NA; AF168751*
GAsr*	NA; AF168752*
ONtr1*	NA; AF168757*
TXki*	NA; AF168759*
TXcc*	NA; AF168758*
TXsc*	NA; AF168760*; DQ529147, DQ529148; DQ529185, DQ529186; DQ529125
NMrg*	NA; AF168756*; DQ529151; DQ529187; DQ529127
MidCon63	ISUA200614; DQ529103; DQ529157, DQ529158; DQ529192; DQ529122
TC34	ISUA200620; DQ529113
TC35	ISUA200621; DQ529112
TC37	ISUA200622; DQ529111
TC38	ISUA200623; DQ529114; DQ529132, DQ529133; DQ529174; DQ529118
TC36	ISUA200625; EU040193; EU040200; EU040201, EU040202; DQ529119
TC45	ISUA200624; EU040194; DQ529134, DQ529135; DQ529175; no data R35
AM7	ISUA200717; EU040181
AMGst18	ISUA200716; EU040186
AMGst6	ISUA200714; EU040187
AMGst1	ISUA200713; EU040188
AMGst2	ISUA200712; EU040195
LT104	ISUA20073; EU040199
SM102	ISUA20075; EU040189
RM46	ISUA20077; EU040193
RM26	ISUA20079; ; EU040184
RM47	ISUA20076; EU040179
RM106	ISUA20074; EU040192
RM15	ISUA200715; EU040185
RC57	ISUA20078; EU040180
LG28	ISUA200718; EU040182
LIRim15	ISUA200710; EU040191
LGa46	ISUA20072; EU040190
LGa45	ISUA20071; EU040196
LGa25	ISUA200711; EU040197
LGaRim17	ISUA200719; EU040198

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Human Recreation and the Nesting Ecology of a Freshwater Turtle (*Chrysemys picta*)

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ABSTRACT. – **Over a 3-year period, we studied the relationship between the intensity of human recreation and the nesting ecology of the painted turtle (*Chrysemys picta*) at a major nesting beach. Our results suggest that the intensity of human recreation at this site had no effect on the decision of turtles to emerge from the water and nest, or on habitat selection by nesting turtles. This apparent lack of effect of human recreation is contrary to the results of many previously published studies on other taxa and underscores the variability in wildlife responses to human recreation and the need for species-specific and population-specific studies.**

The effects of human recreation on wildlife populations have recently received a great deal of scientific attention, in part because of a rapid increase in outdoor recreation activities over the last several decades (Flather and Cordell 1995). To date, most reported effects of recreation and human disturbance on wildlife have been negative (Boyle and Samson 1985; Carney and Sydeman 1999). However, some investigators have suggested that the effects of human disturbance on wildlife populations may be overestimated (Boyle and Samson 1985; Nisbet 2000), and the impact of human recreation on groups such as reptiles is not well studied (Boyle and Samson 1985).

Although declines in populations of organisms such as amphibians (Wake 1991) have been well publicized, concordant declines in turtle populations have received comparatively little attention (Gibbons et al. 2000; Klemens 2000). Although habitat alteration is a major factor in turtle population declines (Mitchell and Klemens 2000), human recreation can also be detrimental (Garber and Burger 1995; Bury and Luckenbach 2002). The potential effects of human disturbance on the nesting ecology of freshwater turtles are significant because females may alter nest-site selection based on the risk that they themselves will be depredated (Spencer 2002; Spencer and Thompson 2003).

If females perceive humans as a predation risk and alter their nesting behavior, maternal and offspring fitness can be altered through combinations of a variety of factors. For example, the site where a female chooses to deposit eggs can affect the probability of nest depredation through nest density (Valenzuela and Janzen 2001; Marchand et al. 2002; but see Burke et al. 1998) and edge effects (Temple 1987; Kolbe and Janzen 2002a). Nest-site selection may also affect offspring survival through temperature-related incubation success (Schwarzkopf and Brooks 1987; Wilson 1998) and overwintering success (Weisrock and Janzen 1999), as well as offspring sex ratio in turtles with temperature-dependent sex determination (reviewed in Bull 1983; Ewert and Nelson 1991; Janzen and Paukstis 1991; Shine 1999).

The purpose of this study was to determine the effects of human recreation on the nesting behavior of the painted turtle (*Chrysemys picta*). In particular, we evaluated how different levels of human recreation on a major nesting