FINAL REPORT

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GENETIC VARIATION WITHIN AND AMONG NATURAL AND CAPTIVE POPULATIONS OF ALLIGATOR SNAPPING TURTLES IN (*Macrochelys temminckii*) OKLAHOMA

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ABSTRACT

The alligator snapping turtle (*Macrochelys temminckii*) is a highly aquatic, freshwater turtle that inhabits forested drainages of the Gulf of Mexico. Declining population trends have been documented across much of its geographic range during the past century, prompting interest in the development of conservation strategies for this species. Developing a sound management plan for *M temminckii* requires an understanding of the genetic diversity and structure of its populations. Recent genetic analyses have shown haplotypic diversity between watersheds across the species' range, indicating limited or non-existent gene flow between watersheds. We assessed the haplotypic diversity for *M. temminckii* between wild populations within the Arkansas and Red river watersheds. Results indicated that all 48 *M. temminckii* examined from Oklahoma possess a previously described haplotype (A). We also used nine bi-parentally inherited microsatellite loci to examine levels of genetic variation within and among populations from eight river drainage basins and two captive populations. Results indicated no statistically significant genetic differentiation between wild populations in Oklahoma, the two captive populations and other populations within the Mississippi drainage basin (F_{ST} = 0.001). Significant population-level separation (F_{ST} = 0.027) was detected in comparisons among separate drainage basins indicating that each drainage basin should serve as a distinct management unit with the Suwannee River basin being the most distinct genetically. Recommendations were made for the conservation of *M. temminckii*.

OBJECTIVE

To assess genetic variation within and among natural and captive populations of alligator snapping turtles.

OVERVIEW

This Final Report includes (1) a brief summary that highlights the research protocol, major results, and management recommendations from this study; (2) Appendix A that summarizes captures and samples used in genetic analyses; and (3) Appendix B that provides a complete account of the research in the form of a M.S. Thesis by Joseph C. Hackler, titled "Assessment of genetic variation within and among natural and captive populations of alligator snapping turtles (*Macrochelys temmincki*)."

INTRODUCTION

The alligator snapping turtle (*Macrochelys temmincki*) is restricted to river drainages of the Gulf of Mexico in the southeastern United States. Anthropogenic factors such as habitat alterations and unregulated harvesting for meat have resulted in the decline of populations of alligator snapping turtles throughout its range (Pritchard 1979, 1989; Roman et al. 1999). In Oklahoma, alligator snapping turtles are restricted to portions of the Arkansas River and Red River watersheds in the eastern quarter of Oklahoma. A private breeder in Perry, Oklahoma, ~~also~~ has a stock of alligator snapping turtles from Missouri that has been offered as a source for restocking alligator snapping turtles in Oklahoma. Similarly, captive alligator snapping turtles bred at Tishomingo National Fish Hatchery could be used for stocking efforts. Because alligator snapping turtles are highly sedentary with females leaving the water only to lay eggs, coupled with the fact that the Arkansas and Red rivers do not connect in Oklahoma, it is possible that alligator snapping turtles in these two rivers represent distinct genetic entities. Such strong genetic differentiation among river drainages has been detected for alligator snapping turtles elsewhere in their range (Roman et al. 1999).

Because (1) previous studies have shown strong genetic differentiation among populations of alligator snapping turtles in different river drainages (Roman et al. 1999) and (2) potential negative impacts of restocking alligator snapping turtles into different river drainages exist if strong genetic differentiation is detected, our purpose was to assess levels of genetic variation within and among river drainages, within the captive population, and between river drainages and the captive population of alligator snapping turtles.

MATERIALS AND METHODS

Alligator snapping turtles were trapped at sites of recent captures of alligator snapping turtles in tributaries occurring in Sequoyah NWR, Little River NWR, the Kiamichi River, and the area around Lake Eufaula in east central Oklahoma (Appendix A). We additionally surveyed areas of the state that historically contained populations of alligator snapping turtles. Upon capture, each individual was marked and a sample of blood was taken following a protocol approved by the Oklahoma State University Animal Care and Use Committee (IACUC Protocol Number AS028). Blood samples were stored in lysis buffer until returning them to Oklahoma State University. In the laboratory, total genomic DNA was extracted using standard protocols (Van Den Bussche et al. 2003). To ascertain levels of genetic variation within and among river drainages and the captive stock, we examined DNA sequence variation in a portion of the mitochondrial genome and allelic variation at 9 nuclear microsatellite loci. For comparative purposes, we included 132 specimens of alligator snapping turtles from the southeastern United States (Appendix B, pages 35–36) that were used in a previous mitochondrial DNA study (Roman et al. 1999). Appendix B provides specific details on procedures and statistical analyses.

RESULTS

Based on the mitochondrial DNA survey, all 48 *M. temminckii* from natural populations in Oklahoma and 7 adults from the Red Rock captive population in Perry, Oklahoma, possessed a single haplotype (haplotype A) as described by Roman et al. (1999) for samples from the Mississippi River and associated drainages (Appendix B). Due to the lack of genetic variation at this maternally inherited marker, no further analyses were performed with the mitochondrial DNA. However, genetic variation at 9 biparentally inherited microsatellite loci was assessed for 245 *M. temminckii*, representing 8 river drainage basins and 2 captive populations (Perry and Tishomingo; Appendix B, Table 2). Overall, genetic differentiation among the 8 river drainage basins (F_{ST} = 0.027) and among the 8 drainage basins and the two captive populations (F_{ST} = 0.026) was statistically significant. With regard to alligator snapping turtles captured and bred in Oklahoma, no statistically significant genetic differentiation was detected between them and any other sampling localities within the Mississippi drainage basin (F_{ST} = 0.001).

MANAGEMENT RECOMMENDATIONS

1. Based on the lack of genetic differentiation among native and captive alligator snapping turtles in Oklahoma, the two captive populations (Perry and Tishomingo) would make good sources for reintroduction in the State and even elsewhere in the Mississippi drainage basin and possibly the Neches drainage basin in Texas.

2. Prior to developing a full-scale headstart program for reintroduction of alligator snapping turtles in Oklahoma or elsewhere, additional work needs to be conducted to address

concerns with captive breeding programs and the release of individuals back into native habitats (Frankham et al. 2002). These include, but are not limited to: minimizing genetic adaptation to captivity, avoiding inbreeding in captive populations and investigating effects of multiple paternity, determining which individuals (e.g., what size and age classes) are appropriate for release, assessing the appropriate number to be released to maximize survival and success of any release, and assessing habitat preferences of headstart turtles to maximize their survival. Failure to address these issues could have serious negative consequences for the success of such a reintroduction program.

3. A sound management plan will need to be developed for alligator snapping turtles that mimics natural levels of gene flow among now fragmented populations due to dams and altered waterways. Because river drainage basins and their associated dams are usually not delineated within a single state's boundaries, development of this management plan will require cooperation among local, state, and federal conservations agencies. Finally, as suggested by the study of Roman et al. (1999) and more convincingly by this study, such a management plan should be developed and employed at a regional level.

LITERATURE CITED

Frankham, R., J. D. Ballou, and D. A. Briscoe. 2002. Introduction to conservation genetics. Cambridge University Press, Cambridge, United Kingdom.

Pritchard, P. C. H. 1979. Encyclopedia of turtles. TFH Publications, Neptune, New Jersey.

Pritchard, P. C. H. 1989. The alligator snapping turtle: biology and conservation. Milwaukee Public Museum, Milwaukee, Wisconsin.

Roman, J., S. D. Santhuff, P. E. Moler, and B. W. Bowen. 1999. Population structure and cryptic evolutionary significant units in the alligator snapping turtle. Conservation Biology 13:135–142.

Van Den Bussche, R. A., S. R. Hoofer, D. A. Wiedenfeld, D. H. Wolfe, and S. K. Sherrod. 2003. Genetic variation within and among fragmented populations of lesser prairie-chickens (*Tympanuchus pallidicnctus*). Molecular Ecology 12:675–683.

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Appendix A

Trapping effort and number of alligator snapping turtles captured by location in 2002 and 2004 (one net night equals one net set overnight for one night). During an earlier study of alligator snapping turtle distribution and ecology supported by Sequoyah NWR in 2002, 45 alligator snapping turtles were captured in 3 counties, and 27 blood samples were collected and used in the genetic analyses reported in this report.

Date	County	Location	Net Nights	Number of Turtles Captured
2002				
29 May	Sequoyah	Big Vian Creek	9	6
30 May	Sequoyah	Big Vian Creek	9	2
4 June	Sequoyah	Big Vian Creek	6	2
6 June	Sequoyah	Hezekiah Creek	6	1
10 June	Muskogee	Dirty Creek	6	1
11 June	Sequoyah	Hezekiah Creek	6	3
17 June	Haskell	Briar Creek	5	1
18 June	Muskogee	Dirty Creek	6	3
20 June	Sequoyah	Big Vian Creek	5	2
24 June	Sequoyah	Big Vian Creek	6	2
25 June	Sequoyah	Little Vian Creek	6	5
26 June	Sequoyah	Big Vian Creek	8	2
9 July	Muskogee	Dirty Creek	9	6
16 July	Sequoyah	Hezekiah Creek	6	0
19 July	Muskogee	Dirty Creek	9	4
20 July	Sequoyah	Little Vian Creek	8	2
28 July	Sequoyah	Big Vian Creek	8	3
2004				
26 May	McIntosh	Mill Creek	8	0
27 May	McIntosh	Mill Creek	8	6
28 May	McIntosh	Mill Creek	8	2
1 June	Pushmataha	Mill Creek	5	0
1 June	Pushmataha	Kiamichi River	4	0
2 June	Pushmataha	Mill Creek	2	0
3 June	Pushmataha	Kiamichi River	9	0
4 June	Pushmataha	Kiamichi River	9	0
11 June	McIntosh	Dutchess Creek WMA	9	0
12 June	McIntosh	Mill Creek	9	2
16 June	McCurtain	Forked Lake	9	0
17 June	McCurtain	Little River	9	0
18 June	McCurtain	Little River	9	0
19 June	McCurtain	Mountain Fork River	5	0

Appendix A Continued.

Date	County	Location	Net Nights	Number of Turtles Captured
21 June	Johnston	McAdam's Pond	4	0
21 June	Johnston	Reeve's Ravine	5	0
22 June	Marshall	Rock Creek	9	0
23 June	Marshall	Rock Creek	9	0
29 June	Pushmataha	Kiamichi River	9	0
30 June	Pushmataha	Kiamichi River	9	0
1 July	Pushmataha	Kiamichi River/Mill Creek	9	1
2 July	Pushmataha	Mill Creek	4	0
8 July	McCurtain	Little River	9	0
9 July	McCurtain	Little River/Mud Creek	9	1
10 July	McCurtain	Little River/Crooked Creek	9	0
11 July	McCurtain	Little River	9	0
12 July	Sequoyah	Little Vian Creek	9	6
13 July	Muskogee	Dirty Creek	9	5
14 July	Atoka	Muddy Boggy Creek	9	0
15 July	Atoka	Muddy Boggy Creek	9	0
5 August	McCurtain	Little River	15	0
6 August	McCurtain	Forked Lake	15	3
7 August	McCurtain	Forked Lake	14	0
Total			395	71 *

*Blood samples for genetic analyses were not available for all captures.

Appendix B

M.S. Thesis by Joseph C. Hackler

Assessment of genetic variation within and among natural and captive populations of alligator snapping turtles (*Macrochelys temmincki*)

ASSESSMENT OF GENETIC VARIATION WITHIN AND AMONG NATURAL AND CAPTIVE POPULATIONS OF ALLIGATOR SNAPPING TURTLES (*MACROCHELYS TEMMINCKII*)

By

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SNAPPING TURTLES

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ASSESSMENT OF GENETIC VARIATION WITHIN AND AMONG NATURAL AND CAPTIVE POPULATIONS OF ALLIGATOR SNAPPING TURTLES (*MACROCHELYS TEMMINCKII*)

ABSTRACT

The alligator snapping turtle (*Macrochelys temminckii*) is a large aquatic species restricted to drainages of the Gulf of Mexico. In recent decades, populations have declined throughout this turtle's range due, in part, to unregulated harvest. With growing interest, managers are now looking to develop protocols for conserving this species. Understanding the genetic diversity and structure of *M. temminckii* populations will assist conservationists in the development of a sound management plan. We assessed haplotypic diversity for *M. temminckii* in Oklahoma. Results indicated that *M. temminckii* in Oklahoma possess a previously described haplotype (A). We also used 9 microsatellite loci to examine levels of within and among population variation for *M. temminckii* from 8 river drainage basins and 2 captive populations. Results indicated significant population-level separation among drainage basins ($F_{ST} = 0.027$) and that drainage basins form distinct management units, with the Suwannee drainage basin being the most distinct genetically and possibly deserving special attention. A sound management plan for *M. temminckii* will require cooperation among local, state, and federal conservation agencies.

INTRODUCTION

The alligator snapping turtle (*Macrochelys temminckii*, Harlan) is a highly aquatic species found in drainages of the Gulf of Mexico in the southeastern United States. Typically a riverine species, it is also found in smaller streams, lakes, oxbows, and bayous (Pritchard 1989). For the most part, only nesting female *M. temminckii* leave the water (Ernst et al. 1994). The alligator snapping turtle is the largest freshwater turtle in North America with wild-caught individuals > 100 kg (Pritchard 1989; Conant and Collins 1998). Due to its large size and susceptibility to trapping, the species has long been harvested for meat, resulting in population declines throughout its range (Pritchard 1989; Sloan and Lovich 1995; Riedle et al. 2005).

In 1983, the U.S. Fish and Wildlife Service was petitioned to list *M. temminckii* as a threatened species, but the petition was denied due to insufficient scientific data regarding population status and trends (Lane and Mitchell 1997). Status of the species was reviewed again in 1991 and 1996 with no further federal action (Lane and Mitchell 1997). At the state level, however, *M. temminckii* is now listed as a species of conservation concern and is afforded some protection by every state within its range (Buhlmann and Gibbons 1997). Current Natural Heritage State Rarity Rankings for *M. temminckii* are as follows (see Appendix A for rank definitions): Alabama S3; Arkansas S4; Florida S3; Georgia S3; Illinois S1; Indiana S1; Iowa SU; Kansas S1; Kentucky S2; Louisiana S3; Mississippi S3; Missouri S2; Oklahoma S2; Tennessee S2S3; and Texas S3 (NatureServe 2005).

To provide data necessary to evaluate the conservation status of *M. temminckii*, Roman et al. (1999) collected blood samples from 158 individuals across 12 drainage

basins from Texas to Florida. Roman et al. sequenced 420 base pairs (bp) of the mitochondrial control region and detected 11 haplotypes separated into eastern, central, and western lineages. Eight of the 11 haplotypes were river-specific. Their results indicated that many river drainages may be distinct management units (MU), and the eastern, central, and western groups may be considered evolutionary significant units (ESUs). Roman et al. (1999) provided a critical first step for proper management of *M. temminckii*, but their data can only be interpreted as demonstrating matrilineal, interdrainage basin, genetic differentiation because they examined only mtDNA. To better understand whether different MUs or ESUs exist within the range of *M. temminckii*, partitioning of genetic variation within and among these 12 drainage basins based on biparentally inherited loci needs to be evaluated.

While Roman et al. (1999) examined *M. temminckii* from a large portion of their range; they did not include samples from Oklahoma, which represents the northwestern extent of the turtle's current distribution. Alligator snapping turtles were once distributed throughout all major river systems in the eastern one-half of Oklahoma (Glass 1940; Webb 1970; Black 1982; Carpenter and Krupa 1989; Heck 1998). However, due to declining numbers of *M. temminckii*, this species has been protected by a statewide closed harvest since 1992 (Levell 1997; Heck 1998). In 1997, the Oklahoma Department of Wildlife Conservation (ODWC) funded a 3-year study to determine the current distribution of *M. temminckii* in Oklahoma. Results of that survey indicated that numbers of *M. temminckii* had declined noticeably throughout most of the state, and current known populations appear to be restricted to a few locations in the eastern one-quarter of the state (Riedle et al. 2005). Sequoyah National Wildlife Refuge (SNWR) in Sequoyah,

Haskell, and Muskogee counties currently harbors the largest known population of *M. temminckii* in Oklahoma. Since 1997, more than 200 individuals have been marked and measured at the refuge (pers. obs.). However, in a recent study, very few *M. temminckii* in other areas of the state were captured (Riedle et al. 2005). Areas of Oklahoma known to still have *M. temminckii* include: Little River, McCurtain County; Mill Creek and Dutchess Creek, McIntosh County; Kiamichi River, Pushmataha County; and Mill Creek, Pushmataha County (Riedle et al. 2005).

There currently is interest in restoring Oklahoma's depauperate populations of *M. temminckii* via captive propagation. Within Oklahoma, there are two captive breeding populations of *M. temminckii*. One population is located at the Tishomingo National Fish Hatchery (TNFH) in Johnston County. The TNFH is currently using 17 turtles from the SNWR as breeding stock and has begun to produce hatchlings for eventual release into areas of historical occurrence. Since 2002, the hatchery has produced nearly 200 hatchlings (Kerry Graves pers. comm.); however, there has yet to be a release of hatchlings into the wild. The second captive population (Red Rock) is located in Noble County and is owned by a private turtle breeder. The breeding stock of this private population comprises turtles purchased from Loggerhead Acres Turtle Farm, Strafford, Missouri. Those turtles purportedly originated from northern Louisiana. During the last four years with permits from the ODWC, more than 250 hatchlings from this private stock have been released into areas of the Tishomingo National Wildlife Refuge (TNWR), Johnston County, Oklahoma (Larry Andrews, pers. comm.). Augmentation of extant wild populations and repatriation of extirpated populations within the species'

historic distribution in Oklahoma with captively reared hatchlings may be a viable management option to restore self-sustaining populations.

Implementation of such a program needs to consider whether the release of hatchlings from a captive population will affect the genetic integrity of wild populations and avoid negative effects associated with outbreeding depression, such as decreased fitness, loss of unique alleles, or inability to maintain local adaptations (Templeton 1994). With the lack of information regarding genetic characteristics of populations of *M. temminckii* in Oklahoma, coupled with no data from biparentally inherited loci throughout the range of *M. temminckii*, it is difficult to assess the impact of releasing captive-bred *M. temminckii* into the wild. It is highly probable that *M. temminckii* in Oklahoma possess haplotype A exhibited by all individuals in the Mississippi River drainage examined by Roman et al. (1999). Moreover, for the same reason, it is highly probable that the turtles used to start the privately owned captive breeding program in Oklahoma also possess haplotype A. However, data are not available to evaluate the genetic uniqueness of *M. temminckii* based on biparentally inherited loci.

Therefore, the objectives of this study were to: 1) assess haplotypic diversity of natural and captive populations of *M. temminckii* in Oklahoma using the same portion of the mitochondrial genome examined by Roman et al. (1999) and 2) assess levels of genetic diversity within and among populations of *M. temminckii* in Alabama, Arkansas, Florida, Louisiana, Mississippi, Missouri, Oklahoma (natural and captive), and Texas based on biparentally inherited microsatellite loci. Addressing these two objectives will provide genetic data for the development of a sound management plan for *M. temminckii* in Oklahoma and elsewhere. Moreover, assessing levels of biparentally inherited genetic

diversity within and among river drainages of *M. temminckii* examined by Roman et al. (1999) will provide additional information for the designation of MUs and ESUs for *M. temminckii* in the southeastern United States.

MATERIALS AND METHODS

Tissue Collection – DNA aliquots of 132 specimens remaining from Roman et al. (1999) were loaned to us for the microsatellite analyses. Those aliquots represented *M. temminckii* from 12 river drainage basins including the Trinity (n = 3), Neches (n = 11), Mississippi (n = 17), Pascagoula (n = 13), Mobile Bay (n = 12), Perdido (n = 1), Pensacola Bay (n = 20), Choctawhatchee (n = 2), Econfina (n = 2), Apalachicola (n = 23), Ochlockonee (n = 10), Suwannee (n = 15), and 3 individuals of unknown origin (see appendix B for locality data). Due to small sample sizes, turtles from the Trinity, Perdido, Choctawhatchee, and Econfina drainage basins were genotyped but excluded from statistical analyses.

Sampling of *M. temminckii* in Oklahoma occurred along tributaries of the Arkansas (n = 43) and Red (n = 5) rivers, both of which are part of the Mississippi River drainage basin (see appendix B for locality data). Oklahoma *M. temminckii* were captured using commercial hoop nets baited with fresh fish. After capture, each individual was marked using a file to make a notch on a posterior marginal scute. Using a pair of veterinarian toenail clippers to clip a toenail just beyond the quick, we collected approximately 250 ul of blood from each turtle. Syringes were used to collect blood from the dorsal cervical sinuses of TNFH hatchlings, representing 5 clutches from 2003 (n = 44). We collected $\leq$ 10% of each animal's blood volume (Oklahoma State University's

IACUC Protocol #AS028). Toenail clips also were used to collect blood from 7 adults from the Red Rock captive population. We also collected tissue from the tails of 25 hatchlings from this population that died naturally after hatching. Clutch assignment and year of hatching were unknown for those turtles. Blood and tissue were stored in 500 ul of lysis buffer (2 M Tris, 0.5 M EDTA, 5 M NaCl, 10% SDS, ddH_2O). Total genomic DNA was extracted using standard protocol (Longmire et al. 1997) and stored in 500 ul of 1 X TE in a refrigerator until needed.

mtDNA – Approximately 420 bp of the $tRNA^{PRO}$ locus and adjoining 5′ end of the mtDNA control region were amplified via standard polymerase chain reaction (PCR) for turtles from natural Oklahoma populations and adults from the Red Rock captive population. Amplifications were conducted in 50-ul reaction volumes using flanking primers developed by Roman et al. (1999). PCR thermal profile consisted of denaturation at 94 °C for 3 min followed by 35 cycles of 94 °C for 1 min, 54 °C for 1 min, and 72 °C for 2 min. Double-stranded amplicons were electrophoresed through a 0.8% agarose gel stained with ethidium bromide and exposed to ultraviolet light for visualization. Successful amplicons were purified using the Wizard PCR Prep DNA Purification System (Promega Corporation, Madison, Wisconsin), and both strands of the amplified products were sequenced using the aforementioned flanking primers and cycle sequencing according to the manufacturer's instructions (Big-DyeTM chain terminators, Applied Biosystems, Inc., Foster City, California). Cycling conditions were as follows: 25 cycles at 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Sequence products were electrophoresed on a 377 Automated DNA Sequencer (Applied Biosystems, Inc., Foster City, California).

AssemblyLIGN 1.0.9 (Oxford Molecular Group PLC 1998) was used to assemble overlapping fragments within individuals, and CLUSTAL X (Thompson et al., 1997) was used to obtain a multiple sequence alignment of all individuals sequenced along with sequences of each haplotype described by Roman et al. (1999). The multiple sequence alignment was imported into MacClade 4.0 (Madison and Madison 2000) to identify variable nucleotide positions.

Microsatellite DNA- Genetic Identification Services (GIS) (Chatsworth, California) constructed four *M. temminckii* genomic libraries. Two of those libraries (A and B) were enriched for trinucleotide microsatellite repeats 'AAT' and 'ATG', respectively; the other two libraries (C and D) were enriched for tetranucleotide microsatellite repeats 'CATC' and 'TAGA', respectively. From those four libraries, GIS developed primer pairs for 10 microsatellite loci: *Mte*A105, *Mte*B103, *Mte*C1, *Mte*C112, *Mte*D2, *Mte*D6, *Mte*D9, *Mte*D106, *Mte*D109, and *Mte*D111 (Table 1). Standard PCR amplifications were performed for each individual for all 10 loci (15-ul reactions consisting each of 9.0 ul of Applied Biosystems True Allele genotyping premix, 3.8 ul of ddH_2O, 1.0 ul of 5.0 uM primer pairs, and 1.2 ul of template DNA). The PCR thermal profile was the same for all loci and consisted of a denaturation and enzyme activation cycle of 95 °C for 12 min followed by 35 cycles of 94 °C for 40 s, 57 °C for 40 s, and 72 °C for 30 s. To ensure that all reactions were completed, a final extension of 72 °C for 4 min was used. Locus *Mte*D6 was excluded from analyses due to lack of confidence in scoring.

Microsatellite variation was visualized primarily using a Perkin-Elmer Applied Biosystems Prism 377 automated sequencer. However, during this project, that machine

was replaced by an Applied Biosystems 3130 Genetic Analyzer. To ensure accuracy in scoring between machines, several individual turtles previously genotyped on the first machine were re-analyzed across all loci with the second machine. Gel images were read by Genescan 3.1 (Applied Biosystems, Inc.), and individuals were genotyped using Genotyper 2.5 (Applied Biosystems, Inc.) and/or GeneMapper 3.7 (Applied Biosystems, Inc.).

Presence of null alleles was evaluated using MICRO-CHECKER (Van Oosterhout et al. 2004). Observed (H_O) and expected (H_E) heterozygosity within populations were calculated using ARLEQUIN 2.0 (Schneider et al. 2000). ARLEQUIN 2.0 also was used to assess deviations from Hardy-Weinberg equilibrium for all locus-population combinations and to test for linkage disequilibrium among loci (Schneider et al. 2000).

ARLEQUIN 2.0 (Schneider et al. 2000) was used to perform analysis of molecular variance (AMOVA) to partition the extent of genetic variation resulting from variation within individuals, within river drainage basins/captive populations, and among river drainage basins/captive populations. We also performed AMOVA for localities within the Mississippi basin, but due to small sample sizes at some localities, samples were lumped into populations based on geographical proximities as follows. All individuals from Louisiana were lumped together. The single individual from Arkansas was combined with individuals from Missouri. Individuals from tributaries of the Red River in Oklahoma were pooled, and individuals from tributaries of the Arkansas River in Oklahoma were considered 2 separate populations: SNWR and Eufaula Reservoir. Using ARLEQUIN, pairwise F_{ST} values were computed among drainage basins. Tests that

involved multiple comparisons were adjusted for a Type I error rate of 5% by the sequential Bonferroni method (Rice 1989).

STRUCTURE 2.0 (Pritchard et al. 2000) was used to obtain Bayesian probabilities of drainage basin membership for each individual based on its genotype, and we compared those results to sampling localities. Using STRUCTURE, we estimated the number of populations (K) by comparing the posterior probabilities (Ln likelihood) for K-values 1–12. Analyses were based on 100,000 Markov chain Monte Carlo iterations after a burn-in period of 50,000 iterations. That analysis detected any population substructure that might be present within river drainage basins and allowed us to assign the 3 individuals of unknown origin to a population along with probability of membership. However, those 3 individuals were not included in any other analyses.

GeneDist, a web-based program developed by J. Brzustowski (http://www2.biology.ualberta.ca/jbrzusto/GeneDist.php), was used to calculate a genetic distance matrix for drainage basins and captive populations. Pairwise genetic distance values (D_S; Nei 1972) were used to generate a neighbor-joining tree using MEGA 2.1 (Kumar et al. 2001).

RESULTS

mtDNA Analyses –All 48 *M. temminckii* from natural populations and 7 adults from the Red Rock captive population in Oklahoma exhibited haplotype A as described by Roman et al. (1999). Due to the lack of haplotypic diversity, no further analyses were performed. Hatchlings from the 2 captive populations were not sequenced because it was

highly likely that they also exhibited haplotype A because all adults from populations that produced them were haplotype A.

Microsatellite Analyses - Genetic variation of 9 microsatellite loci was assessed in 245 *M. temminckii* representing 8 river drainage basins and the 2 captive populations (Table 2). In certain cases, even after repeated attempts, some individuals did not amplify at a locus, and those individuals were omitted for that locus. The highest average observed heterozygosity ($\overline{H}_O$) was observed for locus *Mte*D109 and the lowest was for *Mte*B103. All loci were polymorphic with an average of 9.33 alleles/locus and a range of 6–16. For every locus except one, $\overline{H}_O$ was lower than average expected heterozygosity ($\overline{H}_E$), and this is probably because populations from different drainage basins were pooled for this analysis. Such observed heterozygote deficiencies are expected when disparate genetic populations are pooled to compute expected heterozygosity (Wahlund effect).

With respect to separate turtle populations, $\overline{H}_O$ was lower than $\overline{H}_E$ in all sampled populations (Table 3). This observed heterozygote deficiency again may be explained by the Wahlund effect (pooling various populations within a drainage basis), but not for the Neches and Pascagoula drainages, which were composed of only one sampling locality. Also, as seen below, there was little genetic differentiation among populations of the same drainage basin. The Red Rock captive population had the highest $\overline{H}_O$ while the Suwannee population in Florida had the lowest. Individuals within the Suwannee population were distinctive because they appeared to be fixed for a private allele (178 bp) at locus *Mte*A105. The Suwannee population also possessed alleles that appeared to be

rare in other populations (locus *Mte*C1, 138 bp and locus *Mte*D2, 119 bp). Assumptions of Hardy-Weinberg equilibrium were violated for 1–9 loci in each population.

Overall genetic differentiation among the 8 river drainage basins ($F_{ST} = 0.027$) and among the 8 drainage basins and 2 captive populations ($F_{ST} = 0.026$) was statistically significant (Table 4). Levels of inbreeding in individuals relative to subpopulations ($F_{IS} = 0.030$) and relative to all populations ($F_{IT} = 0.056$) also were statistically significant among drainage basins and among drainage basins and captive populations ($F_{IS} = 0.010$, $F_{IT} = 0.036$; Table 4). The proportion of genetic variation attributable to within-individual variation ($V_c = 94.39\%$) was higher than variation among individuals within drainage basins ($V_b = 2.90\%$) and among drainage basins ($V_a = 2.71\%$). A similar pattern was noted when comparisons were made among drainage basins and captive populations ($V_a = 96.36\%$, $V_b = 1.01\%$, $V_c = 2.64\%$). AMOVA performed on data from sampling localities within the Mississippi drainage basin revealed no genetic differentiation within the drainage basin ($F_{ST} = 0.001$; Table 4).

Pairwise comparisons of F_{ST} inferred significant levels of genetic differentiation between various drainage basins (Table 5). An important finding was the large level of differentiation between the Suwannee drainage basin and all other 7 populations. Mobile Bay and Ochlockonee populations were each significantly differentiated from 5 of the 7 other populations but not as strongly as Suwannee from the other populations.

In the STRUCTURE assessment of number of populations (K), the Ln likelihoods of the fit of the data to K = 1–12 were: K = 1, -6699.3; K = 2, -5490.1; K = 3, -5042.8; K = 4, -4584.9; K = 5, -4237.7; K = 6, -4357.7; K = 7, -3995.1; K = 8, -3987.8; K = 9, -3715.9; K = 10, -3655.2; K = 11, -3648.1; and K = 12, -3650.7. That distribution was

unimodal with the highest probabilities for $K \geq 10$; the small differences among probabilities for $K \geq 10$ suggested that there were rather minor aspects of genetic structure caused by null alleles or deviations from Hardy-Weinberg equilibrium or linkage disequilibrium (Pritchard et al. 2000). Therefore, $K = 10$ discrete populations was chosen following recommendations of J.K Pritchard and W. Wen (Documentation for STRUCTURE Software, http://pritch.bsd.uchicago.edu). Individuals from Mobile Bay, Pascagoula, Pensacola, and Suwannee drainage basins clustered into their own groups. Individuals from Apalachicola and Ochlockonee basins clustered into 1 group. Turtles from the Neches drainage basin clustered with a few individuals from the Mississippi basin and the Red Rock captive population, while the remaining 4 population clusters were composed of different combinations of individuals from the Mississippi basin, Tishomingo captive population, and the Red Rock captive population. Two of the unknown individuals clustered with individuals from the Neches, and the third unknown individual clustered with turtles from Mobile Bay.

In support of results from *F*-statistics and STRUCTURE analyses, pairwise genetic distance values (D_S) calculated for the 8 drainage basins and 2 captive populations revealed significant levels of genetic differentiation, especially regarding the Suwannee basin (Table 6). An unrooted neighbor-joining tree of D_S-values illustrated the close association of alligator snapping turtles of the Neches, Mississippi, and the captive populations, Tishomingo and Red Rock (Figure 2). Also evident was the close association of turtles from the Apalachicola and Ochlockonee basins and the Pascagoula and Mobile Bay basins, and the substantial divergence of *M. temminckii* within the Suwannee basin (Figure 2).

DISCUSSION

Roman et al. (1999) examined mtDNA variation within and among river drainages throughout the range of *M. temminckii* and concluded that this variation corresponded to biogeographic barriers resulting in eastern, central, and western lineages. Moreover, while Roman et al. detected considerable variation among basins, with 8 of the 11 mtDNA haplotypes detected being specific for a river drainage, haplotype A (which corresponded to the western lineage) was detected in 3 drainages (Mississippi, Trinity, and Neches). Given this previous information, it is not surprising that individuals we sampled in Oklahoma possessed haplotype A. We sampled *M. temminckii* in tributaries of the Red and Arkansas Rivers, both part of the Mississippi River drainage basin. It also is not surprising that individuals sampled from the captive breeding programs possessed haplotype A because the sources of these captive breeding populations were from the Mississippi River drainage basin.

Results from microsatellite analyses are concordant with results based on mtDNA (Roman et al. 1999). We detected significant genetic differentiation between river drainage basins ($F_{ST} = 0.027$), and that differentiation was further supported by results of pairwise F_{ST} comparisons and the neighbor-joining tree constructed from pairwise genetic distances among collecting localities. Based on analysis of mtDNA, Roman et al. (1999) concluded that *M. temminckii* within the Neches, Mississippi, Pascagoula, Mobile Bay, and Pensacola drainage basins formed what they termed the western assemblage, turtles within the Apalachicola and Ochlockonee grouped as the central assemblage, and turtles from the Suwannee formed the eastern assemblage. Based on microsatellite data, turtles representing the Mississippi drainage basin are closely aligned with turtles from

the 2 captive populations, which is expected given their origins, and *M. temminckii* from the Neches are closely related to those in the Mississippi. The close assocation between the Apalachicola and Ochlockonee drainage basins also is apparent. Finally, it is clear based on microsatellite data that *M. temminckii* from the Suwannee are highly divergent from turtles in other drainage basins.

Significant genetic differentiation among drainage basins is reflective of the aquatic nature of *M. temminckii*. Several studies have examined movements of *M. temminckii*, yet none of these studies recorded overland movements (Sloan and Taylor 1987; Harrel et al. 1996; Trauth et al. 1998; Riedle et al. 1999). It is thought that only female *M. temminckii* leave the water, and they generally only move a few meters from water to nest and then return to water (Ernst et al. 1994). Thus, movement between drainages would involve swimming downstream into the Gulf of Mexico and then into another drainage. Although *M. temminckii* are capable of exploiting brackish habitats for extended periods of time (Jackson and Ross 1971), movements between drainages in this manner are probably rare. Other possibilities for interdrainage dispersal include major flooding events that temporarily connect adjacent drainage basins and stream captures, although these also are probably rare.

Results suggest a total of 10 genetically distinct populations of *M. temminckii*. However, using no prior information about population membership, the 10 population clusters did not match exactly with the 8 drainage basins and 2 captive populations. Drainage basins that formed independent clusters were Mobile Bay, Pascagoula, Pensacola, and Suwannee. These independent clusters are not surprising given the results from AMOVA and pairwise comparisons. The Apalachicola and Ochlockonee drainage

basins formed a cluster together. Results from Roman et al. (1999) are concordant with these findings. They found that all individuals within the Ochlockonee possessed a single haplotype (H), and that haplotype was the most prominent haplotype within the Apalachicola drainage basin. This may reflect stream capture by the Ochlockonee from the Apalachicola (Gilbert 1987). The remaining population clusters were a mixture of individuals from the Neches and Mississippi drainage basins and the 2 captive populations. This too is not surprising given that all of these individuals possess haplotype A, and both captive populations comprise individuals from tributaries of the Mississippi drainage basin. Because most drainage basins clustered into single independent groups, results from STRUCTURE analysis further suggested there was no population subdivision within drainage basins.

Conservation Implications - Waples (1991) described the concept of the ESU, based on ecological, historical, and genetic uniqueness of the group. Subsequently, Moritz (1994) suggested that an explicit phylogenetic definition would be useful for identifying ESUs and recommended that the criteria for different ESUs are that groups be reciprocally monophyletic for mtDNA sequences and possess significantly different allelic frequencies based on nuclear loci. While most people agree with the concept of recognizing ESUs, the problem has been in determining the best approach for defining ESUs (Cracraft 1997; Crandall et al. 2000), and this has resulted in several alternative definitions. However, given data we have for *M. temminckii* and following the approach of Moritz (1994) for defining ESUs, mtDNA and microsatellite data support the original conclusions of Roman et al. (1999) that the western, central, and eastern lineages represent ESUs. Based on results from this study and the study by Roman et al. (1999),

the three groups originally identified by Roman et al. as eastern, central, and western are best recognized as ESUs in that our study has shown that these three groups have significant differences in allelic frequencies. It is quite obvious that the Suwannee drainage basin is quite different from other populations and should receive special conservation priority. Alligator snapping turtles within this basin have a highly divergent endemic haplotype (K; Roman et al. 1999); they exhibit large and significant pairwise F_{ST}-values with all other populations; they are fixed for a private allele at one microsatellite locus (*Mte*A105) and possess alleles at other loci that appear to be rare in other drainage basins. Considering the totality of the evidence, *M. temminckii* within the Suwannee are truly unique and are probably best recognized as a cryptic species or, at the very least, a subspecies whose genetic diversity should be of paramount importance for conservation.

Captive propagation and reintroduction of headstarted *M. temminckii* have been proposed as a method for reestablishing depleted populations where suitable habitat is still available. Based on our results and those of Roman et al. (1999), we conclude that drainage basins form distinct management units within the broader groupings (ESUs) of western, central, and eastern assemblages. Captive populations should be created with individuals that encompass the entire genetic diversity present within each drainage basin and/or ESU.

Oklahoma already has two established captive populations of *M. temminckii*. Our results indicate that these populations would make a good source for reintroduction into the Mississippi drainage basin and possibly the Neches drainage basin. However, allelic diversity and overall genetic diversity of these captive populations should be increased to

encompass the entire genetic diversity within those drainage basins. Reintroductions are already occurring in Oklahoma using hatchlings from the privately owned captive population. Since 2001, more than 250 hatchlings from this captive population have been released into areas of TNWR. Thus, results of this study reaffirm that captive breeding programs for *M. temminckii* could be established within each of the major river drainages. However, before headstart breeding programs are created for *M. temminckii*, several issues regarding the captive breeding program need to be addressed, such as, minimizing genetic adaptation to captivity, developing methods to avoid inbreeding in captive populations, the potential occurrence of multiple paternity in captive breeding groups, when to release headstarted individuals, the appropriate number of individuals to be released at each location, and what size/age ensures the best chance of survival. Also, a study addressing habitat preferences of headstarted individuals is much needed to ensure that suitable habitat is available for released individuals.

A sound management plan for *M. temminckii* will require cooperation between local, state, and federal conservation agencies. One particular problem that will have to be solved is fragmentation due to dams. The Mississippi was the only drainage basin with large enough sample sizes distributed throughout the region to allow testing for further population subdivision. Results from AMOVA for the Mississippi drainage basin revealed no significant genetic differentiation among sampling localities (F_{ST} = 0.001). Before construction of dams along the Mississippi River and its tributaries, there probably was gene flow throughout the entire drainage basin. Several mark-recapture studies have shown that *M. temminckii* can make movements of several kilometers within a few months (Shipman 1993; Harrel et al. 1996; Trauth et al. 1998). With life spans that

extend beyond 50 years (Pritchard 1989), an alligator snapping turtle moving several kilometers per month could travel great distances within a drainage basin over its lifetime. Gene flow is now disrupted among localities within the drainage basin because of dams, effectively cutting them off from one another. However, dams have not been in place long enough for the sampling localities to diverge significantly from one another. Because the generation time of *M. temminckii* can be 11–21 years (Dobie 1971; Tucker and Sloan 1997), most dams have been disrupting gene flow for only a few generations. With more time, differentiation between population fragments will increase due to genetic drift. This could lead to a loss of overall genetic diversity for *M. temminckii* within the Mississippi drainage basin and elsewhere. Dams have stopped gene flow that may have occurred historically within drainage basins. Therefore, any management plan for *M. temminckii* should consider mimicking natural levels of gene flow between now fragmented populations, and because river drainage basins are usually not delineated within a single state's boundaries, a conservation strategy should be developed and employed at a regional level.

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LITERATURE CITED

Black, J.H. 1982. An annotated bibliography to articles, notes and photographs on reptiles and amphibians appearing in Oklahoma Game and Fish News, Oklahoma Wildlife, and Outdoor Oklahoma. Oklahoma Herpetological Society Special Publication Number 2.

Buhlmann, K.A. and J.W. Gibbons. 1997. Imperiled aquatic reptiles of the southeastern United States: Historical review and current conservation status. Pp. 201–231, *in* G.W. Benz and D.E. Collins (eds.). Aquatic fauna in peril: the southeastern perspective. Special publication 1, Southeast Aquatic Research Institute, Lenz Design and Communications, Decatur, GA.

Carpenter, C.C. and J.J. Krupa. 1989. Oklahoma Herpetology: An annotated bibliography. University of Oklahoma Press, Norman, OK.

Conant, R. and J.T. Collins. 1998. A field guide to reptiles and amphibians of eastern and central North America. Third edition, expanded. Houghton Mifflin Company, New York, NY.

Cracraft, J. 1997. Species concepts in systematics and conservation biology: an ornithological viewpoint. Pp. 325–339, *in* M.F. Claridge, H.A. Dawah, and M. R. Wilson (eds.). Species: The Units of Biodiversity. Chapman & Hall, London.

Crandall, K.A., O.R.P. Bininda-Edmonds, G.M. Mace, and R.K. Wayne. 2000. Considering evolutionary processes in conservation biology: an alternative to "evolutionary significant units. Trends in Ecology and Evolution 15:290–295.

Dobie, J.L. 1971. Reproduction and growth in the alligator snapping turtle, *Macroclemys temminckii* (Troost). Copeia 4:645–658.

Ernst, C.H., J.E. Lovich, and R.W. Barbour. 1994. Turtles of the United States and Canada. Smithsonian Institution Press, Washington, D.C.

Gilbert, C.R. 1987. Zoogeography of the freshwater fish fauna of southern Georgia and peninsular Florida. Brimleyana 13:25–54.

Glass, P.B. 1949. *Macroclemys temminckii* in Oklahoma. Copeia 2:138–141.

Harrel, J.B., C.M. Allen, and S.J. Herbert. 1996. Movements and habitat use of subadult alligator snapping turtles (*Macroclemys temminckii*) in Louisiana. American Midland Naturalist 135:60–67.

Heck, B.A. 1998. The alligator snapping turtle, *Macroclemys temmincki*, in southeastern Oklahoma. Proceedings of the Oklahoma Academy of Science 78:53–58.

Jackson, C.G., Jr. and A. Ross. 1971. The occurrence of barnacles on the alligator snapping turtle, *Macroclemys temminckii* (Troost). Journal of Herpetology 5:188–189.

Kumar, S., K. Tamura, I.B. Jakobsen, and M. Nei. 2001. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics 17:1244–1245.

Lane, J.J. and W.A. Mitchell. 1997. Species profile: alligator snapping turtle (*Macroclemys temminckii*) on military installations in the southeastern United States. Technical Report SERDP-97-9, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Levell, J.P. 1997. A field guide to reptiles and the law. Second revised edition. Serpent's Tale Natural History Book Distributors, Lanesboro, MN.

Longmire, J.L., M. Maltbie, and R.J. Baker. 1997. Use of 'lysis buffer' in DNA isolation and its implications for museum collections. Occasional Papers, The Museum, Texas Technical University 163:1–3.

Madison, D.R. and W.P. Madison. 2000. MacClade 4.0. Sinauer Associates Inc. Publishers, Sunderland, MA.

Moritz, C.C. 1994. Defining "evolutionary significant units" for conservation. Trends in Ecology and Evolution 9:373–375.

NatureServe. 2005. NatureServe explorer: an online encyclopedia of life [web application]. Version 4.6. NatureServe, Arlington, Virginia. Available http://www.natureserve.org/explorer. (Accessed: November 22, 2005).

Nei, M. 1972. Genetic distance between populations. American Naturalist 106:283–291.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 142:1061–1064.

Pritchard, P.C.H. 1989. The alligator snapping turtle: biology and conservation. Milwaukee Public Museum, Milwaukee, WI.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Riedle, J.D., P.A. Shipman, S.F. Fox, and D.M. Leslie Jr. 2005. Status and distribution of the alligator snapping turtle, *Macrochelys temminckii*, in Oklahoma. Southwestern Naturalist 50:79–84.

—. 1999. Status, distribution and habitat use of the alligator snapping turtle in Oklahoma. Final Report Federal Aid Project E-4, Oklahoma Department of Wildlife Conservation, Oklahoma City, OK. 34 pp.

Roman, J., S.D. Santhuff, P.E. Moler, and B.W. Bowen. 1999. Population structure and cryptic evolutionary units in the alligator snapping turtle. Conservation Biology 13:135–142.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin: a software for population genetic analysis. Version 2.000. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Shipman, P.A. 1993. Natural history of the alligator snapping turtle (*Macroclemys temminckii*) in Kansas. Kansas Herpetological Society Newsletter 93:14–17.

Sloan, K.N. and J.E. Lovich. 1995. Exploitation of the alligator snapping turtle, *Macroclemys temminckii*, in Louisiana: a case study. Chelonain Conservation and Biology 1:221–222.

Sloan, K.N. and D. Taylor. 1987. Habitats and movements of adult alligator snapping turtles in northeast Louisiana. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 41:343–348.

Templeton, A.R. 1994. Coadaptation, local adaptation, and outbreeding depression. Pp. 152–153, *in* G.K. Meffe and R.K. Carroll (eds.). Principles of Conservation Biology. Sinauer Associates Inc. Publishers, Sunderland, MA.

Thompson, J.D., T.J. Gibson, F. Plewnik, F. Jeanmaugin, and D.G. Higgins. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24:4876–4882.

Trauth, S.E., J.D. Wilhide, and A. Holt. 1998. Population structure and movement patterns of alligator snapping turtles in northeastern Arkansas. Chelonian Conservation and Biology 3:64–70.

Tucker, A.D. and K.N. Sloan. 1997. Growth and reproductive estimates from alligator snapping turtles, *Macroclemys temmicnkii*, taken by commercial harvest in Louisiana. Chelonian Conservation and Biology 2:587–592.

Van Oosterhaut, C., W.F. Hutchinson, D.M. Willis, and P. Shipley. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535–538.

Waples, R.S. 1991. Pacific Salmon, *Oncorhynchhus* spp., and the definition of a species under the Endangered Species Act. Marine Fisheries Review 53:11–22.

Webb, R.G. 1970. Reptiles of Oklahoma. University of Oklahoma Press, Norman, OK.

Table 1. - Primer sequences for 10 microsatellite loci of alligator snapping turtles.

Locus	Sequence
Mte A105 F	5' - TGC TCA GGG AGA TTA GAG AGG - 3'
Mte A105 R	5' - AGG ATT ATG TTT TCC AAT GTG C - 3'
Mte B103 F	5' - GCA AAG TGT TTA GCC CTA TG - 3'
Mte B103 R	5' - CCA GGA TGA CAA CCA CAG - 3'
Mte C1 F	5' - CGT CAC ACC TCC CCT CTT AG - 3'
Mte C1 R	5' - CTC CTG TCC CGA TTT TTC AC - 3'
Mte C112 F	5' - TTA CCT GCT CAT CTA CCA ACT C - 3'
Mte C112 R	5' - AAA GAA AGA GAA GGG TGT GTG - 3'
Mte D2 F	5' - CAC CTC TCC AGA TGG CAT TAG - 3'
Mte D2 R	5' - AAA AAC TAC CCC ACC CTC AAC - 3'
Mte D6 F	5' - TGC TGT ATT CTG AGT GGT AAT G - 3'
Mte D6 R	5' - ACA CAG TCA ATG CTG CTA GAG - 3'
Mte D9 F	5' - CCA GAT GCT AGT CTC ACA CC - 3'
Mte D9 R	5' - GCT TAC TGG AAT TAA CCT CAT G - 3'
Mte D106 F	5' - TTA TGG GAA AGG GTT ATT AGC - 3'
Mte D106 R	5' - GCG AAA AGG AAG GTT TAT G - 3'
Mte D109 F	5' - CCT CCC CCC ATA GAT AAA ATA C - 3'
Mte D109 R	5' - ACT GGT TAG CAA CTC CAA CTT C - 3'
Mte D111 F	5' - TCC ACA AAC TCC CAT CTT C - 3'
Mte D111 R	5' - CCA CAC GGA AAA ATC TAT CTA C - 3'

Table 2. - Genetic variation assessed at 9 microsatellite loci for alligator snapping turtles including the number of alleles (A), sample size (n), and average observed ($\overline{H}_O$) and average expected ($\overline{H}_E$) heterozygosity.

Locus	A	n	$\overline{H}_O$	$\overline{H}_E$
Mte A105	7	244	0.291	0.434
Mte B103	9	237	0.112	0.558
Mte C1	7	244	0.413	0.500
Mte C112	8	243	0.263	0.391
Mte D2	6	243	0.179	0.369
Mte D9	11	244	0.490	0.551
Mte D106	7	240	0.352	0.544
Mte D109	16	245	0.633	0.610
Mte D111	13	244	0.414	0.546
Overall	9.33	243	0.350	0.500

Table 3. - Genetic variation assessed at 9 microsatellite loci for 8 natural and 2 captive populations of alligator snapping turtles in the southeastern United States. Column labels are identical to those in Table 2.

Population	*n*	$\overline{H}_O$	$\overline{H}_E$	Average no. alleles/locus	Total no. alleles
Neches	11	0.414	0.576	3.67	33
Mississippi	65	0.345	0.572	5.67	51
Pascagoula	13	0.407	0.535	3.22	29
Mobile Bay	12	0.231	0.468	3.22	29
Pensacola	20	0.413	0.600	4.44	40
Apalachicola	23	0.364	0.582	4.44	40
Ochlockonee	10	0.433	0.514	1.78	16
Suwannee	15	0.082	0.237	1.89	17
Tishomingo	44	0.313	0.343	2.67	24
Red Rock	32	0.542	0.557	4.00	36

Table 4. - Analyses of molecular diversity across 9 microsatellite loci for alligator snapping turtles.

Comparison	F - statistic	Significance level
Drainage basins as separate populations	$F_{IS} = 0.030$	$p < 0.001$
	$F_{ST} = 0.027$	$p < 0.001$
	$F_{IT} = 0.056$	$p < 0.001$
Drainage basins and captives as separate populations	$F_{IS} = 0.010$	$p = 0.009$
	$F_{ST} = 0.026$	$p < 0.001$
	$F_{IT} = 0.036$	$p < 0.001$
Localities within Mississippi River basin as separate populations	$F_{IS} = 0.011$	$p = 0.007$
	$F_{ST} = 0.001$	$p = 1.000$
	$F_{IT} = 0.012$	$p = 0.106$

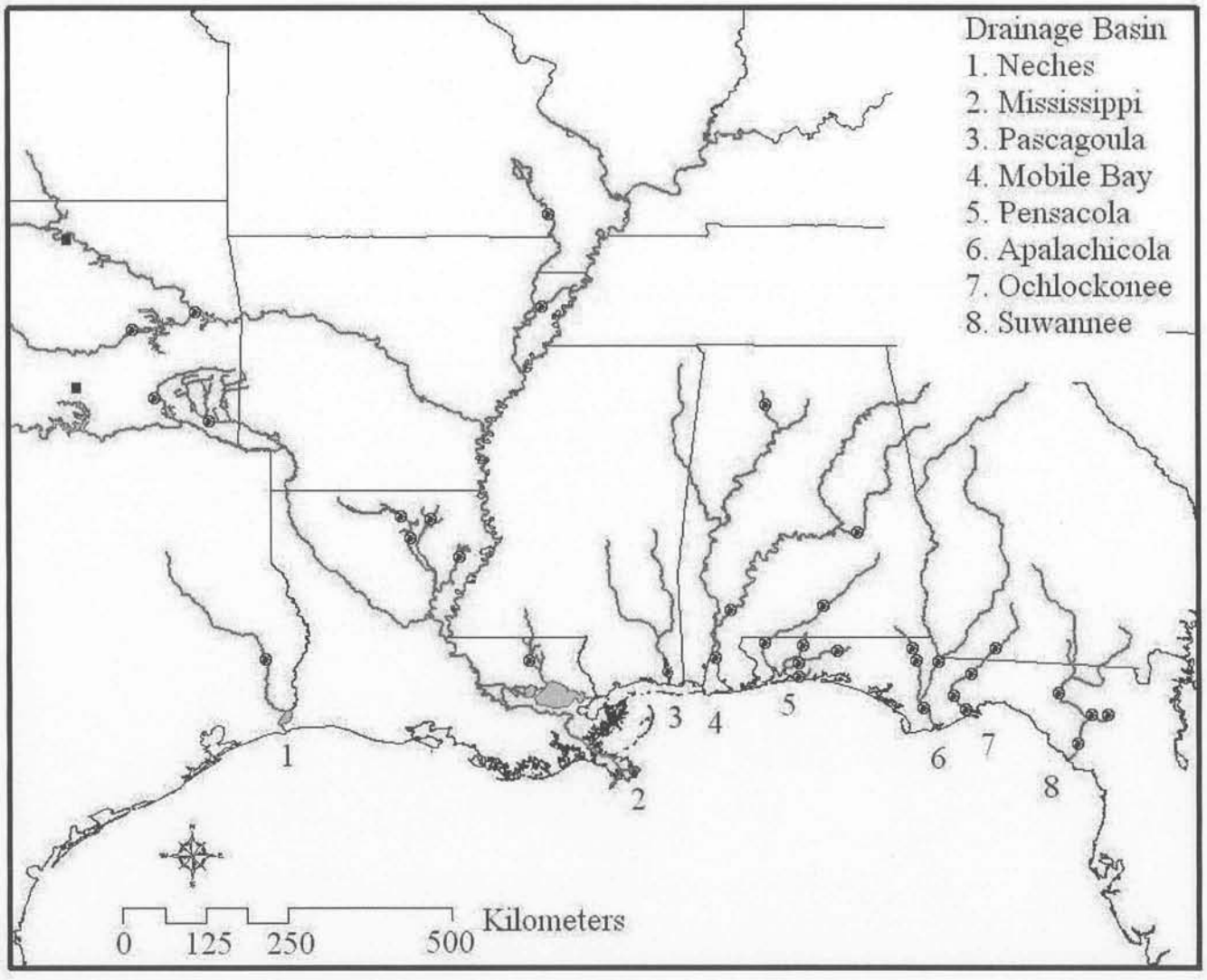
Table 5. - Pairwise F_{ST}-values obtained from 9 microsatellite loci for 8 natural populations of alligator snapping turtles in the southeastern United States.

Population	Neches	Mississippi	Pascagoula	Mobile Bay	Pensacola	Apalachicola	Ochlockonee	Suwannee
Neches	-							
Mississippi	0.002	-						
Pascagoula	0.000	0.002	-					
Mobile Bay	0.009	0.010*	0.009*	-				
Pensacola	0.001	0.002	0.001	0.010*	-			
Apalachicola	0.000	0.002	0.000	0.009*	0.001	-		
Ochlockonee	0.010*	0.012*	0.010*	0.020	0.011*	0.009*	-	
Suwannee	0.136*	0.121*	0.134*	0.144*	0.129*	0.127*	0.148*	-

* indicates significant values after sequential Bonferroni correction.

Table 6. - Matrix of genetic distance values (D_S) obtained via genotypes from 9 microsatellite loci for 8 natural and 2 captive populations of alligator snapping turtles in southeastern United States.

Population	Neches	Miss	Pascag	Mobile	Pensa	Apalach	Ochlock	Suwan	Tish	Red Rock
Neches	-									
Mississippi	0.396	-								
Pascagoula	0.758	0.703	-							
Mobile Bay	0.682	0.700	0.245	-						
Pensacola	0.686	0.946	0.702	0.767	-					
Apalachicola	0.518	0.840	0.921	1.003	0.619	-				
Ochlockonee	0.693	1.168	1.528	1.543	0.940	0.125	-			
Suwannee	3.257	2.375	2.614	1.834	2.147	1.623	1.770	-		
Tishomingo	0.666	0.174	1.305	1.212	1.354	1.198	1.460	1.768	-	
Red Rock	0.358	0.279	0.722	0.656	1.145	1.047	1.424	1.905	0.298	-


Drainage Basin
1. Neches
2. Mississippi
3. Pascagoula
4. Mobile Bay
5. Pensacola
6. Apalachicola
7. Ochlockonee
8. Suwannee
1
2
3
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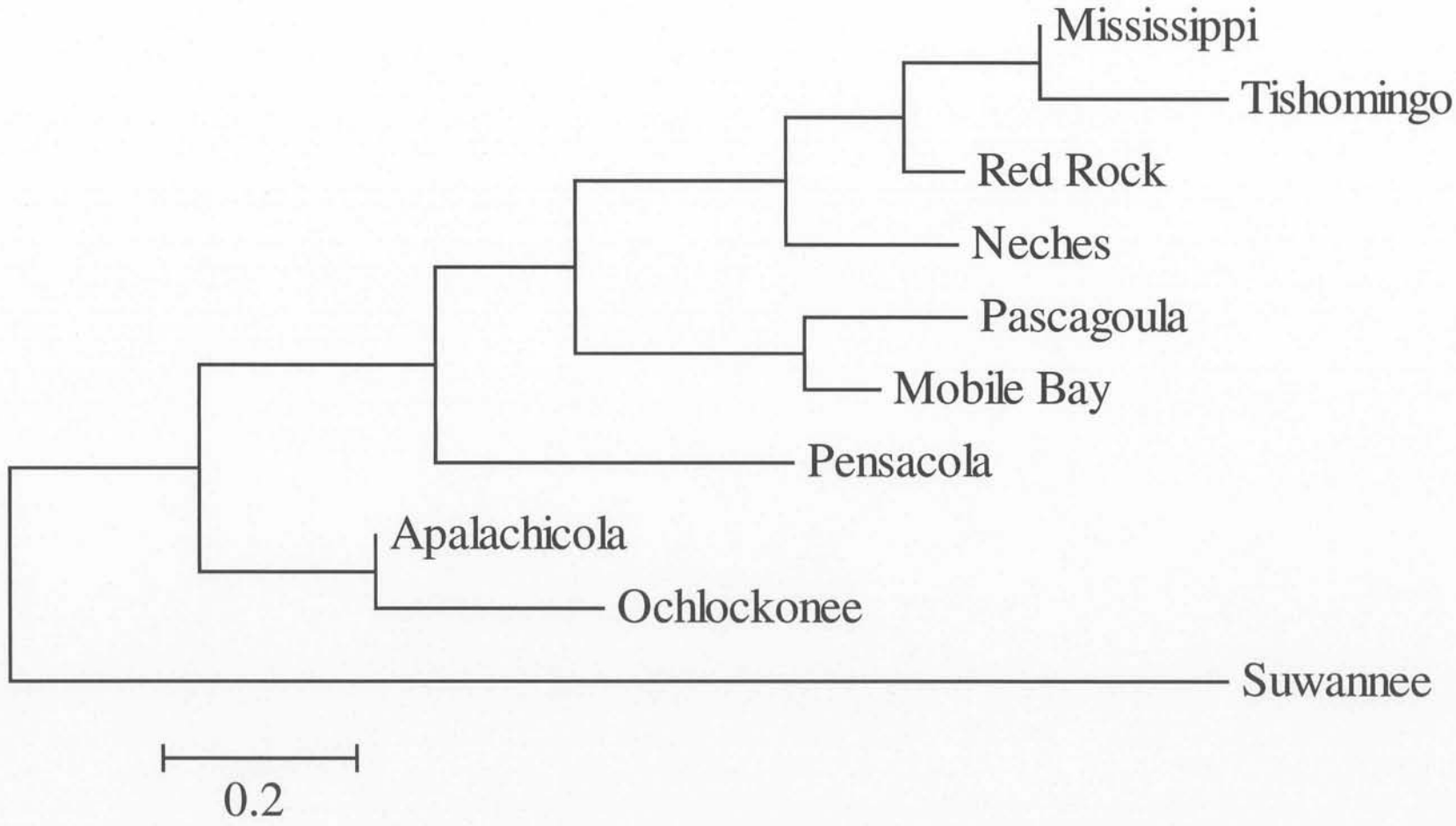
0 125 250 500 Kilometers


Mississippi
Tishomingo
Red Rock
Neches
Pascagoula
Mobile Bay
Pensacola
Apalachicola
Ochlockonee
Suwannee
0.2

Appendix A. Natural Heritage State Rarity Rank Definitions. Two codes together represent an inexact range (e.g., S1S2).

S1 = Critically imperiled in the state because of extreme rarity or other factors making it especially vulnerable to extirpation from the state. (Typically 5 or fewer occurrences or very few remaining individuals or acres)

S2 = Imperiled in the state because of rarity or other factors making it very vulnerable to extirpation from the state. (Typically 6 to 20 occurrences or few remaining individuals or acres)

S3 = Rare or uncommon in the state. (Typically 21 to 100 occurrences)

S4 = Widespread, abundant, and apparently secure in state, with many occurrences, but the taxon is of long-term concern. (Usually more than 100 occurrences)

SU = Uncertain. Possibly in peril in the state, but status is uncertain. More information is needed.

Appendix B. Collection locales and number of individuals sampled.

Drainage Basin	Site	Number of Individuals
Trinity	Bedias Creek, Madison-Leon Counties, TX	3
Neches	Bingham Lake, Tyler County, TX	11
Mississippi	Captive from Cache River, AR	1
	Tensas River, Madison Parish, LA	1
	Bayou Gallion, Morehouse Parish, LA	2
	Bayou Desiard, Ouachita Parish, LA	2
	Bayou D'Arbonne, Union Parish, LA	1
	Black River, Butler County, MO	10
	Kiamichi River/Mill Creek, Pushmataha County, OK	1
	Mud Creek, Little River NWR, McCurtain County, OK	1
	Little Vian Creek, Sequoyah NWR, Sequoyah County, OK	8
	Dirty Creek, Sequoyah NWR, Muskogee County, OK	12
	Forked Lake, Little River NWR, McCurtain County, OK	3
	Lake Eufaula/ Mill Creek, McIntosh County, OK.	10
	Big Vian Creek, Sequoyah NWR, Sequoyah County, OK	8
	Hezekiah Creek, Sequoyah NWR, Sequoyah County, OK	4
	Briar Creek, Sequoyah NWR, Haskell County, OK	1
Pascagoula	Jackson County, MS	13
Mobile Bay	Bear Creek, Baldwin County, AL	3
	Southern Delta, Baldwin County, AL	2
	Turkey Creek, Baldwin County, AL	5
	Tallapoosa River, Macon County, AL	1
	Lost Creek at Townley, Walker County, AL	1
Perdido	Styx River, Baldwin County, AL	1
Pensacola	Conecuh River, above Gantt Dam, Covington-Crenshaw Counties, AL	4
	Escambia River, north of SR 4, Escambia County, FL	4

Drainage Basin	Site	Number of Individuals
Pensacola	East Bay River, Eglin AFB, Okaloosa County, FL	3
	Shoal River, above US 90, Okaloosa County, FL	3
	Yellow River, Eglin AFB, Okaloosa County, FL	2
	Yellow River, south of CR 2, Okaloosa County, FL	1
	Blackwater River, below Blackwater River State Park, Santa Rosa County, FL	2
	Escambia River, Santa Rosa County, FL	1
Choctawhatchee	Holmes Creek, Washington County, FL	2
Econfina	Blue Springs, north of SR 20, Washington County, FL	2
Apalachicola	Apalachicola River, north of SR 20, Calhoun County, FL	18
	Chipola River, north of Florida Taverns State Park, Jackson County, FL	1
	Chipola River, north of I-10, Jackson County, FL	3
	Captive taken from Chipola River	1
Ochlockonee	Revell Landing, Liberty County, FL	1
	Near Old Bainbridge Road, Leon County, FL	2
	Whitehead Landing, Liberty County, FL	2
	Porter Lake, at Forest Highway 13, Liberty County, FL	2
	Wakulla County, FL	3
Suwannee	Santa Fe River, below Olustee Creek, Alachua County, FL	1
	New River, Union County, FL	1
	Santa Fe River, south of 121, Union-Alachua Counties, FL	5
	Santa Fe River, near Highway 18, Alachua County, FL	2
	Santa Fe River, Worthington Springs, Union County, FL	1
	Suwannee River, Dixie County, FL	3
	Suwannee River, Dowling Park, Suwannee County, FL	2

VITA

Joseph C. Hackler

Candidate for the degree of

Master of Science

Thesis: ASSESSMENT OF GENETIC VARIATION WITHIN AND AMONG NATURAL AND CAPTIVE POPULATIONS OF ALLIGATOR SNAPPING TURTLES (*MACROCHELYS TEMMINCKII*)

Major Field: Zoology

Biographical:

Personal Data: Born in Tulsa, Oklahoma, on May 9, 1976, the son of Ralph and Judy Hackler.

Education: Graduated from Bartlesville High School, Bartlesville, Oklahoma in May 1994; received Computer Aided Drafting degree from Tri County Area Vocational-Technical School in May 1994; received Bachelor of Science degree in Zoology from Oklahoma State University, in Oklahoma in May, 2002; completed the requirements for Master of Science Degree in Zoology at Oklahoma State University in May, 2006.

Experience: Seasonal field technician for the U.S. Fish and Wildlife Service, 2000-2002; Oklahoma State University, Department of Zoology graduate teaching assistant, fall semester 2002-fall semester 2005; Field technician for Environmental Management at Tinker AFB, Oklahoma, 2003; Graduate research assistant for the Oklahoma Cooperative Fish and Wildlife Research Unit June-July, 2004-2005; Field coordinator assistant for U.S. Army Corps of Engineers Research Laboratory, 2006-present.

Professional Memberships: American Society of Ichthyologists and Herpetologists, Southwestern Association of Naturalists, Oklahoma Academy of Sciences

Name: Joseph C. Hackler Date of Degree: May 2006

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: ASSESSMENT OF GENETIC VARIATION WITHIN AND AMONG NATURAL AND CAPTIVE POPULATIONS OF ALLIGATOR SNAPPING TURTLES (*MACROCHELYS TEMMINCKII*)

Pages in Study: 36 Candidate for the degree of Master of Science

Major Field: Zoology

Scope and Method of Study: The alligator snapping turtle (*Macrochelys temminckii*) is a large aquatic species restricted to drainages of the Gulf of Mexico. In recent decades, populations have declined throughout this turtle's range due, in part, to unregulated harvest. With growing interest, managers are now looking to develop protocols for conserving this species. Understanding the genetic diversity and structure of *M. temminckii* populations will assist conservationists in the development of a sound management plan. We assessed haplotypic diversity for *M. temminckii* in Oklahoma. We also used 9 microsatellite loci to examine levels of within and among population variation for *M. temminckii* from 8 river drainage basins and 2 captive populations.

Findings and Conclusions: Results indicated that alligator snapping turtles in Oklahoma possess a previously described haplotype (A). Results also indicated that there were significant population-level separations among drainage basins ($F_{ST} = 0.027$) and that drainage basins form distinct management units, with the Suwannee drainage basin being the most distinct genetically and possibly deserving special attention. A sound management plan for alligator snapping turtles is going to require cooperation between local, state, and federal conservation agencies.

ADVISOR'S APPROVAL: Dr. Stanley F. Fox