Zootaxa 3786 (2): 141–165
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3786.2.4
http://zoobank.org/urn:lsid:zoobank.org:pub:06D44DCE-0816-459A-A201-171F273BC210

Taxonomic assessment of Alligator Snapping Turtles (Chelydridae: *Macrochelys*), with the description of two new species from the southeastern United States

TRAVIS M. THOMAS[1,4,8], MICHAEL C. GRANATOSKY[2,4], JASON R. BOURQUE[3], KENNETH L. KRYSKO[4], PAUL E. MOLER[5], TONY GAMBLE[6], ERIC SUAREZ[1], ERIN LEONE[5], KEVIN M. ENGE[5] & JOE ROMAN[7]

[1]*Department of Wildlife Ecology & Conservation, University of Florida, Gainesville, FL 32611, USA.*
E-mails: travis.thomas@myfwc.com; eric.suarez@myfwc.com
[2]*Animal Locomotion Laboratory, Department of Evolutionary Anthropology, Duke University, Durham, NC 27708, USA.*
E-mail: michael.granatosky@duke.edu
[3]*Division of Vertebrate Paleontology, Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA.*
E-mail: jbourque@ufl.edu
[4]*Division of Herpetology, Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA.*
E-mail: kenneyk@ufl.edu
[5]*Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, Gainesville, FL 32601, USA.*
E-mails: paul.moler@myfwc.com; erin.leone@myfwc.co; kevin.enge@myfwc.com
[6]*Department of Genetics and Cell Biology & Development, University of Minnesota, Minneapolis, MN 55455, USA.*
E-mail: gambl007@umn.edu
[7]*Gund Institute for Ecological Economics, Rubenstein School of Environment and Natural Resources, University of Vermont, Burlington, VT 05405, USA. E-mail: joe.roman@uvm.edu*
[8]*Corresponding author*

Abstract

The Alligator Snapping Turtle, *Macrochelys temminckii*, is a large, aquatic turtle limited to river systems that drain into the Gulf of Mexico. Previous molecular analyses using both mitochondrial and nuclear DNA suggested that *Macrochelys* exhibits significant genetic variation across its range that includes three distinct genetic assemblages (western, central, and eastern = Suwannee). However, no taxonomic revision or morphological analyses have been conducted previously. In this study, we test previous hypotheses of distinct geographic assemblages by examining morphology, reanalyzing phylogeographic genetic structure, and estimating divergence dating among lineages in a coalescent framework using Bayesian inference. We reviewed the fossil record and discuss phylogeographic and taxonomic implications of the existence of three distinct evolutionary lineages. We measured cranial (n=145) and post-cranial (n=104) material on field-captured individuals and museum specimens. We analyzed 420 base pairs (bp) of mitochondrial DNA sequence data for 158 *Macrochelys*. We examined fossil *Macrochelys* from ca. 15–16 million years ago (Ma) to the present to better assess historical distributions and evaluate named fossil taxa. The morphological and molecular data both indicate significant geographical variation and suggest three species-level breaks among genetic lineages that correspond to previously hypothesized genetic assemblages. The holotype of *Macrochelys temminckii* is from the western lineage. Therefore, we describe two new species as *Macrochelys apalachicolae* sp. nov. from the central lineage and *Macrochelys suwanniensis* sp. nov. from the eastern lineage (Suwannee River drainage). Our estimates of divergence times suggest that the most recent common ancestor (MRCA) of *M. temminckii* (western) and *M. apalachicolae* (central) existed 3.2–8.9 Ma during the late Miocene to late Pliocene, whereas *M. temminckii*-*M. apalachicolae* and *M. suwanniensis* last shared a MRCA 5.5–13.4 Ma during the mid-Miocene to early Pliocene. Examination of fossil material revealed that the fossil taxon *M. floridana* is actually a large *Chelydra*. Our taxonomic revision of *Macrochelys* has conservation and management implications in Florida, Georgia, and Alabama.

Key words: *Macrochelys*, Chelydridae, Morphology, Conservation, Fossil, Genetics, Phylogeography, Suwannee River, Apalachicola River

Accepted by U. Fritz: 12 Feb. 2014; published: 9 Apr. 2014

Introduction

The Alligator Snapping Turtle, *Macrochelys temminckii* (Troost *in* Harlan 1835), is the largest freshwater turtle in North America and restricted to river systems that drain into the northern Gulf of Mexico from Texas to Florida (Pritchard 2006). Both observational and telemetry data suggest that terrestrial dispersal and movement are rare (Sloan & Taylor 1987; Harrell *et al.* 1996), and the restriction of *M. temminckii* to riverine habitats has subsequently led to geographic and genetic isolation (Roman *et al*. 1999; Echelle *et al*. 2010). Previous examination of both mitochondrial (mtDNA) and nuclear DNA (nDNA) among *M. temminckii* populations revealed significant genetic variation across their range (Roman *et al*. 1999; Echelle *et al*. 2010). Based on mtDNA, Roman *et al*. (1999) hypothesized the existence of three distinct genetic assemblages: western, central, and eastern = Suwannee. Based on microsatellite data, Echelle *et al*. (2010) suggested recognizing six Evolutionarily Significant Units (ESUs) among *M. temminckii* populations ((1) Trinity, Neches, and Mississippi, (2) Pascagoula, (3) Mobile and Perdido, (4) Pensacola, (5) Choctawhatchee, Econfina, Apalachicola, and Ochlockonee, and (6) Suwannee), which coincided with assemblages found by Roman *et al*. (1999); however, Roman *et al*. (1999) used a more conservative definition of ESU and grouped the Trinity, Neches, Mississippi, Pascagoula, Mobile and Perdido, and Pensacola into one "western" assemblage.

Both molecular studies indicated limited genetic exchange among populations inhabiting different river drainages (Roman *et al*. 1999; Echelle *et al*. 2010). Using an arbitrary strict molecular clock (1.2-2.4% per million years taken from green sea turtles [*Chelonia mydas*]) and parsimony analysis, Roman *et al*. (1999) suggested that the three genetic assemblages last shared a common ancestor during the late Pliocene to early Pleistocene. However, this estimated divergence should be interpreted with caution, as assumptions of strict molecular clock estimates are often unrealistic (Drummond *et al*. 2006). To date, no morphological analyses or taxonomic revision has been conducted. In this study, we test the existing hypothesis that there are distinct genetic assemblages by examining morphology, reanalyzing phylogeographic genetic structure, conducting divergence dating estimates among lineages in a coalescent framework, reviewing the fossil record, and discussing phylogeographic and taxonomic implications of distinct evolutionary lineages among the different assemblages.

Methods

Morphological analysis. Adult *Macrochelys temminckii* (field-captured animals and museum specimens) were examined from throughout the geographic range (Figure 1; Appendix). Because scutes were intact on most museum specimens and field-captured animals, we measured observable characteristics found on the carapace. Representative data from recent field captures were deposited as vouchers in the Florida Museum of Natural History (FLMNH), University of Florida Herpetology collection (UF). Additional museum specimens were examined from the Chelonian Research Institute (CRI) and Tulane University Museum of Natural History (TU), as were photographs of a specimen from the Muséum National d'Histoire Naturelle de Paris (MNHNP).

Morphological examination of osseous features consisted of cranial (n=145) and post-cranial (n=104) material. We analyzed photographs of the holotype (MNHN-AZ-AC-A4540) of *M. temminckii* housed at MNHNP. Prior to analyses, specimens were classified as belonging to either the western, central, or eastern (Suwannee) assemblage based on locality data and the molecular groupings of Roman *et al*. (1999) (Appendix). For the skull, an angular measurement of the squamosal bone was obtained by first photographing a lateral view of the cranium and then measuring the squamosal angle (SQA) from the image. (Figure 2). A metric scale (mm) was included in all images to allow accurate measurements. Straight-line skull length (TSL) and straight-line skull width (TSW) (Figure 2) were also measured in order to examine the effect of skull size on variability in SQA via regression. Analysis of variance was used to test for morphological differences among assemblages. Univariate analyses were conducted in SigmaStat ver. 3.5 (Aspire Software International, Ashburn, VA). Digital images were analyzed with the software ImageJ (Schneider et al., 2012; http://rsbweb.nih.gov/ij/download.html).

Post-cranial measurements included straight-line caudal notch width (CNW), straight-line caudal notch depth (CND), and caudal notch area (CNA) (Figure 3). As with skulls, measurements were collected from photographs using ImageJ. We adjusted for error among photographs by taking the mean of measurements from three separate images of each specimen, and this was used for all subsequent analyses. An analysis of covariance was used to test

for morphological differences among geographic assemblages for each measurement, and carapace length (CL) was included as a continuous covariate (SAS v9.3, Cary, NC).

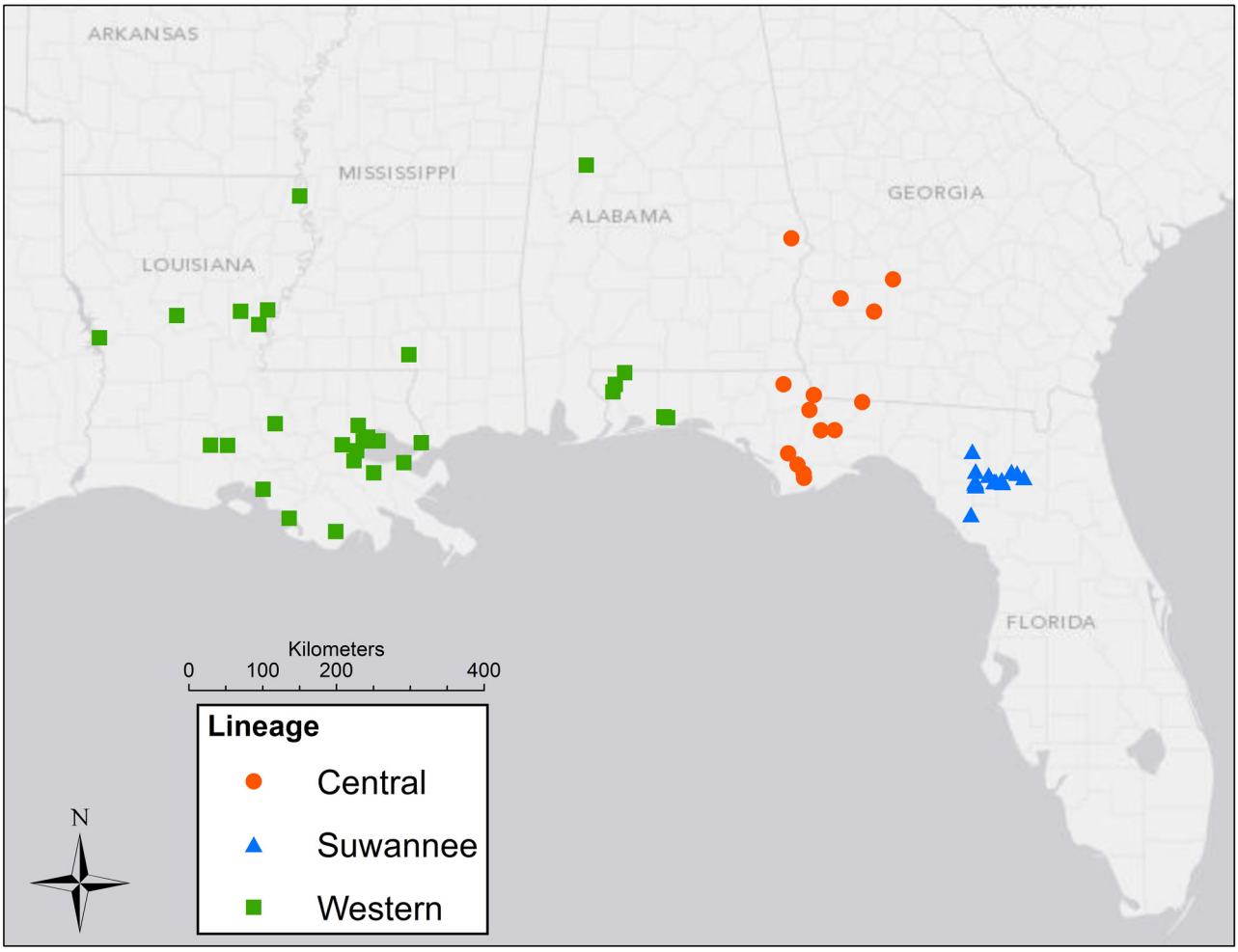


FIGURE 1. Map of sampling localities of *Macrochelys* used for morphological analyses. Multiple specimens were often collected from the same localities.

We sought to explore whether carapace morphology could be used to distinguish individuals from three genetic assemblages. To test this, we used a principal component analysis (PCA) to summarize the osteometric data. PCA was the preferred multivariate technique because the goal was to explore variation in the metric data as well as to examine the distribution of sample taxa in multidimensional morphospace (Neff & Marcus 1980; de Queiroz & Good 1997). Measurements included in the PCA consisted of CNW, CND, and CNA. To account for the effects of body size variation in this sample, each post-cranial measurement from a specimen was divided by the carapace length of that specimen prior to analysis. To normalize the data for each sample, each linear variable included in the PCA was log transformed to create a log shape variable (Jungers *et al*. 1995). Shapiro-Wilk and Levene's tests were conducted to assure normality and equality of variances for the data of each sample. PCA was conducted using a correlation matrix in SAS v9.3 (Cary, NC).

Molecular analyses. Representative mtDNA sequence data consisting of two partial genes (tRNA and the 5′ end of the control region) were downloaded from GenBank for 158 *Macrochelys temminckii* (Reference Sequences: AF056522–AF056524; *see* Roman 1999; Roman *et al*. 1999), along with one each of the following outgroup taxa: *Chelydra serpentina* (GenBank: AF029986.1; Walker *et al*. 1998; Roman 1999) and *Chelonia mydas* (NCBI Reference Sequence: NC_000886.1; Kumazawa & Nishida 1999). These representative *M. temminckii* sequences were then compared to Roman (1999) to determine sequence data and construct a DNA matrix. A total of 420 base pairs (bp) of sequence data was analyzed, including 367 bp for control region and 53 bp for $tRNA^{Pro}$.

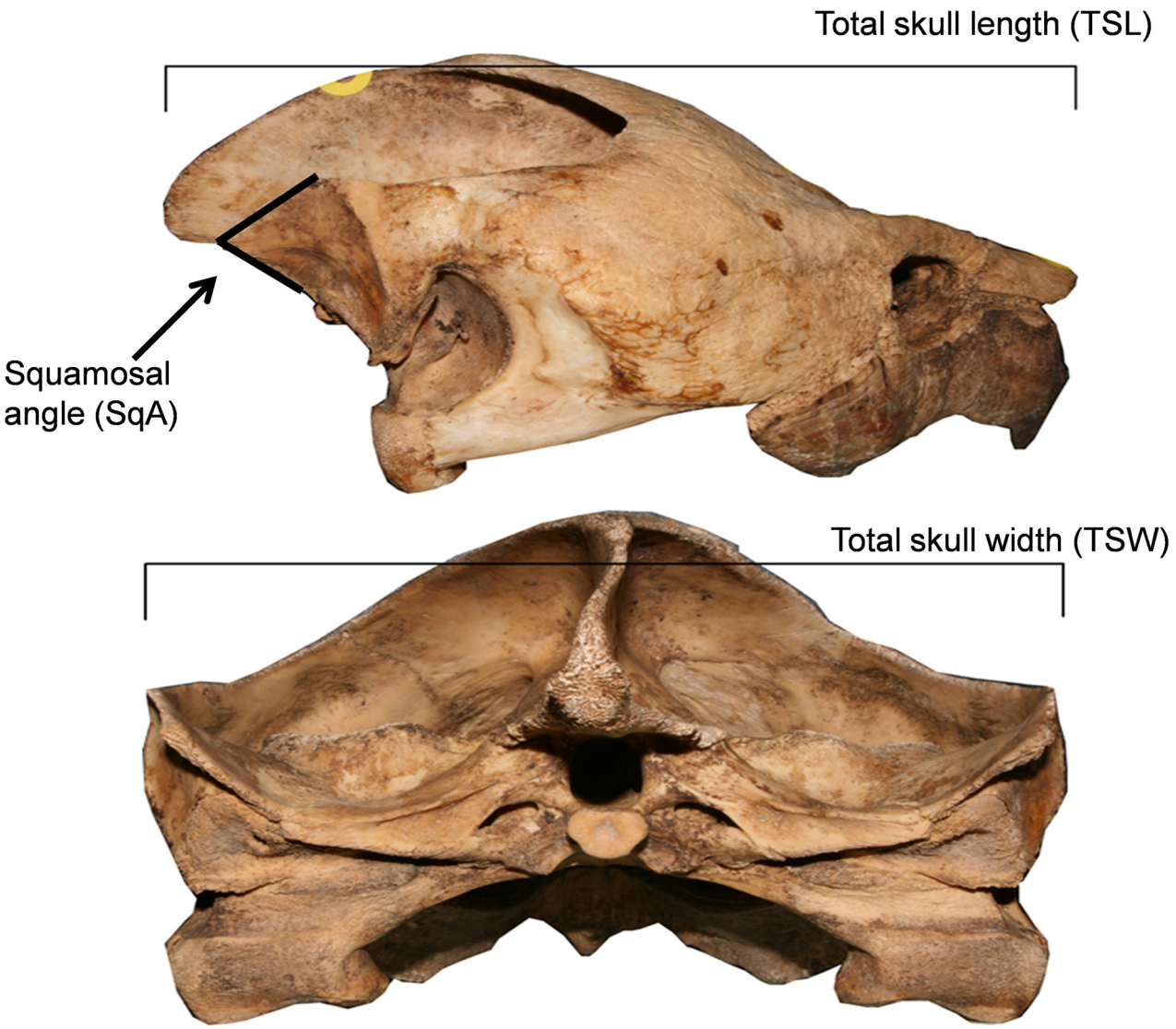


FIGURE 2. Cranial measurements used in present study to quantify shape variation among the three lineages of extant *Macrochelys*.

Phylogenetic inference, divergence dating, and demographic analyses. Sequence comparisons for the number of informative characters and unique haplotypes were obtained using DnaSP (ver. 5.10.01; Rozas 2009). Relationships among mtDNA samples were estimated in a coalescent framework using Bayesian inference (BI) in BEAST version 1.6.1 (Drummond & Rambaut 2007; Drummond *et al*. 2009). All sample sequences were included in analyses even if they were a redundant haplotype, which is necessary to estimate unbiased population parameters and divergent times in a coalescent framework (Kuhner 2009). The best-fit nucleotide substitution model was that of Hasegawa, Kishino and Yano (1985), with gamma distributed rate heterogeneity (HKY + Γ) as determined using the corrected Akaike Information Criterion (AICc) obtained from jModelTest (ver. 0.1.1; Posada 2008). A relaxed phylogenetics method was used to infer each lineage without relying on an arbitrary molecular clock (Zuckerkandl & Pauling 1965) that incorporates uncertainty in the tree estimation process. An uncorrelated exponential relaxed clock (Aris-Brosou & Yang 2002; Drummond *et al*. 2006) with a coalescent tree model, constant population size, and UPGMA starting tree were used as priors.

Fossil calibrations are crucial in divergence dating analyses, because it is not possible to estimate absolute ages from molecular data alone (Weinstock *et al*. 2005; Ho & Phillips 2009). Fossil data included into a molecular data set in the form of parametric distributions offer a high degree of flexibility in integrating a time scale (i.e., an estimate and statistical confidence interval for a divergence time of an evolutionary event) into a phylogenetic

analysis (Morrison 2008; Ho & Phillips 2009). An exponential prior on the minimum age of the most recent common ancestor (MRCA) of *Chelydra* and *Macrochelys* was set to 17.5 Million years ago (Ma) using the oldest known *Macrochelys* fossil, *M. schmidti* (*see* Hutchison 2008). *Macrochelys schmidti* is known from the Marsland Formation (now thought to consist of a composite of both the Running Water Formation at 17.5–19 Ma and Anderson Ranch Formation or Upper Harrison Bed at 19–19.5 Ma) (Tedford *et al*. 2004) in the early Hemingfordian North American Land-Mammal Age (NALMA) (ca. 16–18.9 Ma) of the Miocene. We used a secondary calibration (the MRCA of Chelydridae and *Chelonia mydas*) to constrain the age of the root of the tree, following the estimate (normal prior mean = 87.0; s.d. = 2) after Near *et al*. (2005).

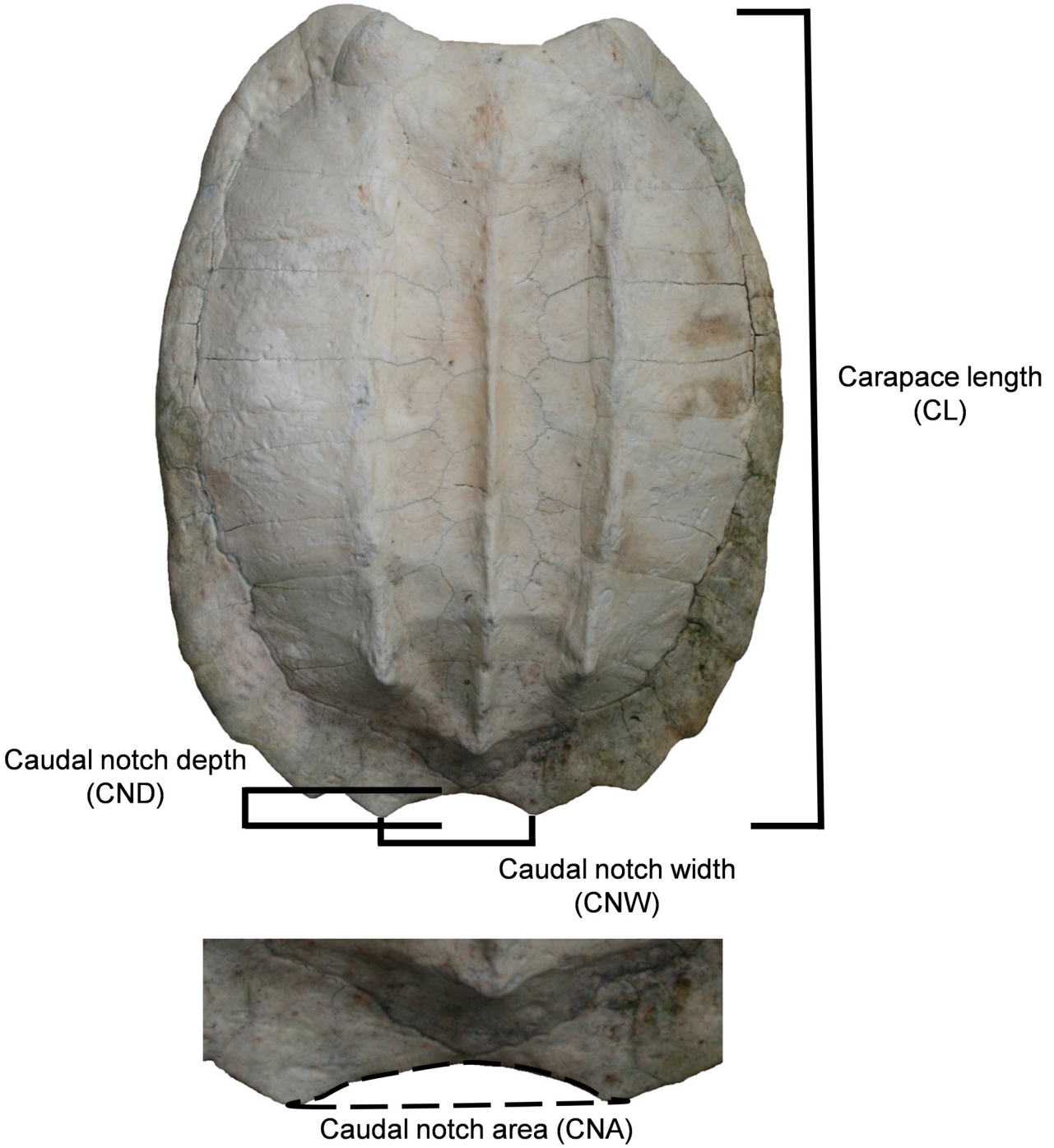


FIGURE 3. Post-cranial measurements used in present study to quantify shape variation among the three lineages of extant *Macrochelys.*

We performed three independent runs for 20 million generations, sampling every 1000 generations. We analyzed *Markov Chain Monte Carlo* (MCMC) runs independently (to confirm chains were converging and not sampling local optima) using Tracer version 1.5 for ESS values >200, as well as for a split standard deviation less than 0.005 for -lnL tree values among chains that indicate parameter stationarity was achieved. Trees sampled prior to stationarity were discarded as burn-in, which occurred prior to 1.5 million generations. Trees from independent MCMC runs were combined and burn-in removed using LogCombiner version 1.5.4. The maximum sum of clade credibilities tree with mean heights was obtained using TreeAnnotator version 1.5.4, and visualized with FigTree version 1.3.1. Nodes were considered strongly supported when the posterior probability (Pp) was greater than 95% (Hillis & Bull 1993; Felsenstein 2004).

The exponential growth rate (g) for populations in the western and central assemblages was estimated using BEAST. Demographic parameters were not estimated for the eastern (Suwannee) lineage because it contained only a single haplotype. Model parameters were the same as the previous BEAST analyses. Two replicate analyses were run for both lineages for 10 million generations, sampling every 1000 generations. Separate runs were combined to estimate the mean value of g with 95% confidence intervals using Tracer. When $g > 0$, population size has been increasing; when $g = 0$, population size is stable; and when $g < 0$, population size is decreasing. As a conservative estimate, if the 95% confidence intervals include zero, it is assumed that population sizes were stable.

General mixed Yule Coalescent. Lohse (2009) suggested the generalized mixed yule coalescent (GMYC) may overestimate species numbers, but others have suggested this is not a major concern in real data sets (Papadopoulou *et al.* 2009; Reid & Carstens 2012). Although the GMYC has some limitations, it is still a valuable tool to identify potential species (Talavera 2013). Species delimitation was performed with the GMYC model implemented in SPLITS (Pons *et al*. 2006; Ezard *et al*. 2009). The GMYC model estimates the number of phylogenetic clusters by identifying the transition between intra- and inter-specific branching patterns on an ultrametric phylogeny of unique haplotypes (Pons *et al*. 2006). A likelihood ratio test was conducted to determine if the model with a shift in the branching processes provided a better fit to the data than the null model lacking a shift in branching processes. The BI ultrametric tree from the BEAST analyses (above) was used for the GMYC analysis after being pruned of redundant haplotypes and the *Chelonia* outgroup.

Species delimitation. Species is a fundamental unit of biology, and accurate delimitation is important (Wiens & Servedio 2000). Despite disagreements, systematic biologists have come to a general agreement that species are lineages (Wiens 2004; de Queiroz 2007; Shaffer & Thomson 2007; Wiens 2007). Combining both molecular and morphological evidence has been recommended to identify lineages and delimit species (Wiens 2002; Dayrat 2005; DeSalle 2005; Shirley 2014). Our study used multiple lines of evidence to address the taxonomy of *Macrochelys*.

Fossil record. We examined fossil *Macrochelys* from ca. 15–16 Ma to the present to better assess historical distributions. Previously reported (Auffenberg 1957) and extralimital records (Meylan 1995) were examined, as well as numerous additional fossils housed in the Division of Vertebrate Paleontology Collection at the FLMNH. The type specimens and unreported material of the Hemphillian taxon *Macrochelys auffenbergi* were examined to better distinguish this fossil taxon from extant *Macrochelys*.

Results

Morphological analysis. Summary statistics for relative values of extant *Macrochelys* skull and carapace measurements are presented in Table 1, and distributions for squamosal angle, caudal notch depth, width, and area can be found in Figure 4. Assemblages differed significantly for SqA ($F_{2,140} = 53.59$, $P < 0.001$), CNW ($F_{2,99} = 7.84$, $P < 0.001$), CND ($F_{2,101} = 17.74$, $P < 0.001$), and CNA ($F_{2,99} = 8.69$, $P < 0.001$).

Regression analyses revealed no significant relationship between SqA and TSL ($P = 0.325$) or TSW ($P = 0.148$). SQA differed significantly ($P < 0.001$) among all three genetic assemblages, with acute and sharp squamosal projections in the Suwannee assemblage and more obtuse and globular squamosal projections in the western and central assemblages (Figure 5). The holotype of *M. temminckii* fell within the western lineage (Figure 6).

Carapace morphology also varies among the three assemblages, as each assemblage possesses a unique caudal notch shape that can be easily observed in both living and preserved specimens (Figure 7). Turtles from the Suwannee assemblage have a very wide, lunate caudal notch, the western assemblage has a much narrower and more wedge-shaped caudal notch, and the central assemblage is somewhat intermediate in shape.

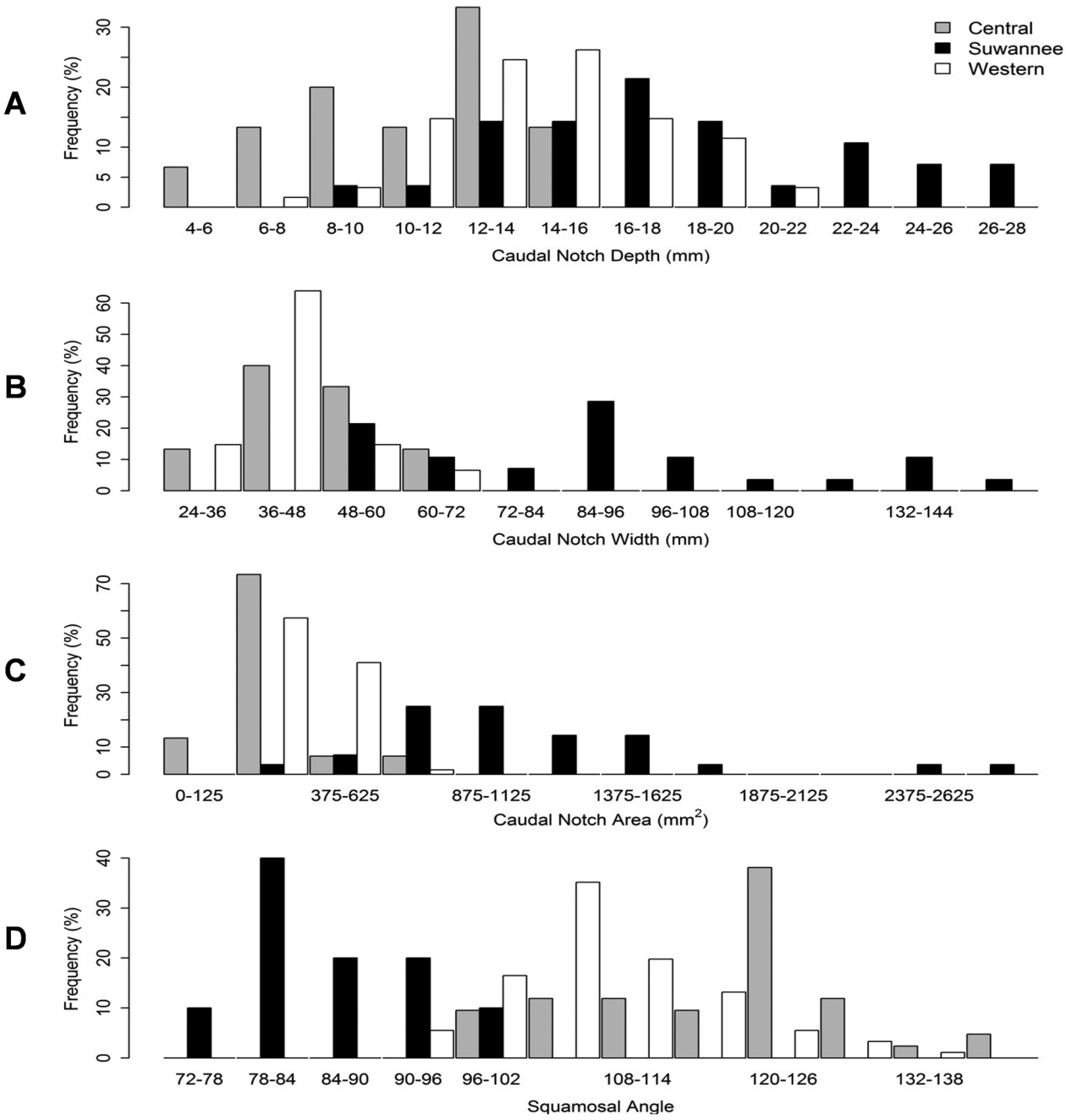


FIGURE 4. Distributions for caudal notch depth, width, area, and squamosal angle for the three *Macrochelys* lineages.

Results from PCA performed on carapace morphology are shown in Figure 8. Together, the first two principal components account for 99.6% of the variance within the sample. The first principal component axis accounts for 78.6% of the variance and separates turtles from the eastern (Suwannee) assemblage from the western and central assemblages. To some extent, this axis separates the western and central assemblages, but there is overlap between these two groups. Turtles from the eastern assemblage (Suwannee) have high scores on this axis and are characterized by relatively wide caudal notches. The second principal component axis accounts for 20.9% of the variance and mainly separates the western assemblage from the other two geographic groups, although there is overlap. This axis primarily correlates with CND. Animals with high scores on this axis have relatively deep CND.

Phylogenetic inference, divergence dating, and demographic analyses. The BI analyses (Figure 9) recovered three major genetic lineages within extant *Macrochelys*: 1) western lineage consisting of samples (n = 93) from the Mississippi, Mobile Bay, and Neches drainages; 2) central lineage consisting of samples (n = 47) from the Apalachicola, Choctawhatchee, Econfina Creek, and Ochlockonee drainages; and 3) eastern (Suwannee)

lineage consisting of samples (n = 18) from the Suwannee drainage. Our estimated divergence times suggest that the MRCA between the western and central lineages occurred about 5.9 Ma (95% Highest Posterior Density [HPD] = 3.2–8.9 Ma) during the late Miocene to mid-Pliocene, whereas the western and central lineages last shared a MRCA with the eastern (Suwannee) lineage about 9.6 Ma (95% HPD = 5.5–13.4 Ma) during the mid to late Miocene. Divergences within each of these major lineages occurred less than 3.5 Ma. Estimates of the exponential growth rates (g) for the western (g = -65.52 [-441.53–205.68]) and central lineages (g = 83.21 [-2872.79–8543.24]) suggest stable population sizes over time.

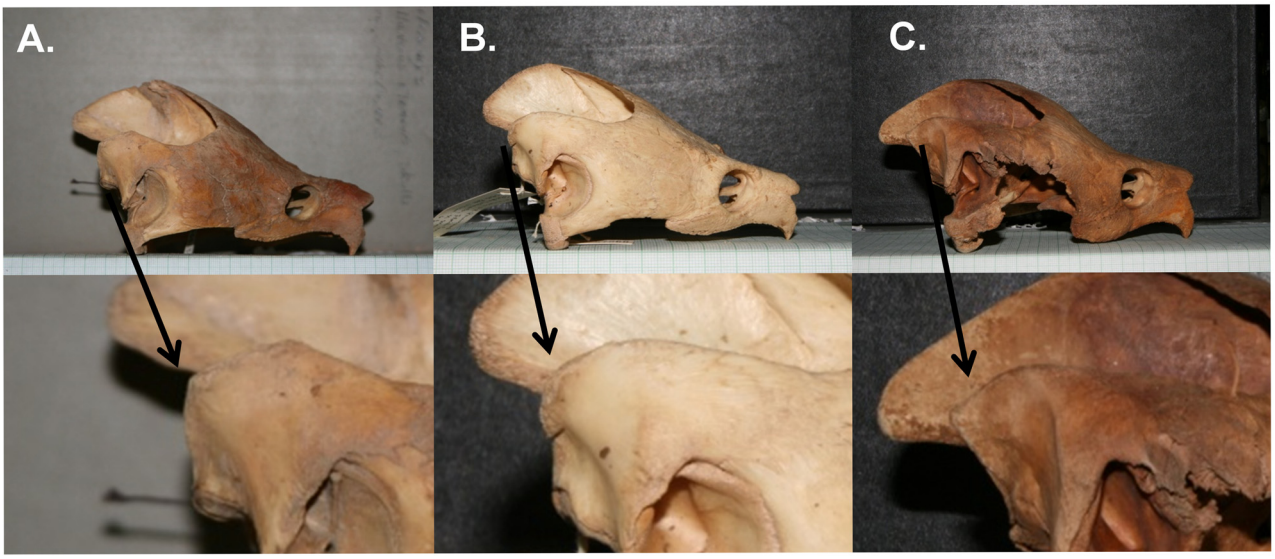


FIGURE 5. Variation of the squamosal in the western (A; TU 17991), central (B; UF 57968), and Suwannee (C; UF 12694) lineages of *Macrochelys*.

TABLE 1. Comparisons of morphometric characteristics for the three *Macrochelys* assemblages (means ± standard error of the mean, lower confidence limit, upper confidence limit).

Lineage	Caudal notch width (mm)			Caudal notch depth (mm)		
	Mean ± SEM	Lower	Upper	Mean ± SEM	Lower	Upper
Western	45.8 ± 1.81[b]	42.2	49.4	14.6 ± 0.49[b]	13.7	15.6
Central	47.0 ± 3.50[b]	40.1	54.0	10.7 ± 0.94[c]	8.8	12.5
Suwannee	76.1 ± 2.82[a]	70.5	81.7	17.7 ± 0.76[a]	16.2	19.2

TABLE 1. (Continued)

Lineage	Caudal notch area (mm^2)			Squamosal Angle
	Mean ± SEM	Lower	Upper	Mean ± SEM
Western	379.3 ± 36.23[b]	307.4	451.2	108.0 ± 0.98[b]
Central	289.6 ± 69.90[b]	150.9	428.3	118.8 ± 1.44[a]
Suwannee	892.8 ± 56.40[a]	780.9	1004.7	85.90 ± 2.95[c]

[abc] Means within characteristics sharing any common letters are not significantly different ($P > 0.05$)

General mixed Yule Coalescent. Three species-level clusters were recovered by the GMYC analysis that correspond with the western, central, and eastern (Suwannee) assemblages of extant *Macrochelys*. The likelihood ratio test was nonsignificant (likelihood of null model = -9.423809, GMYC model = -8.362642, P = 0.5474074). Therefore, the model with a shift in the branching processes did not provide a better fit to the data than the null model lacking a shift. Our phylogenetic inference indicated the presence of the same three separately evolving lineages found by Roman *et al*. (1999).

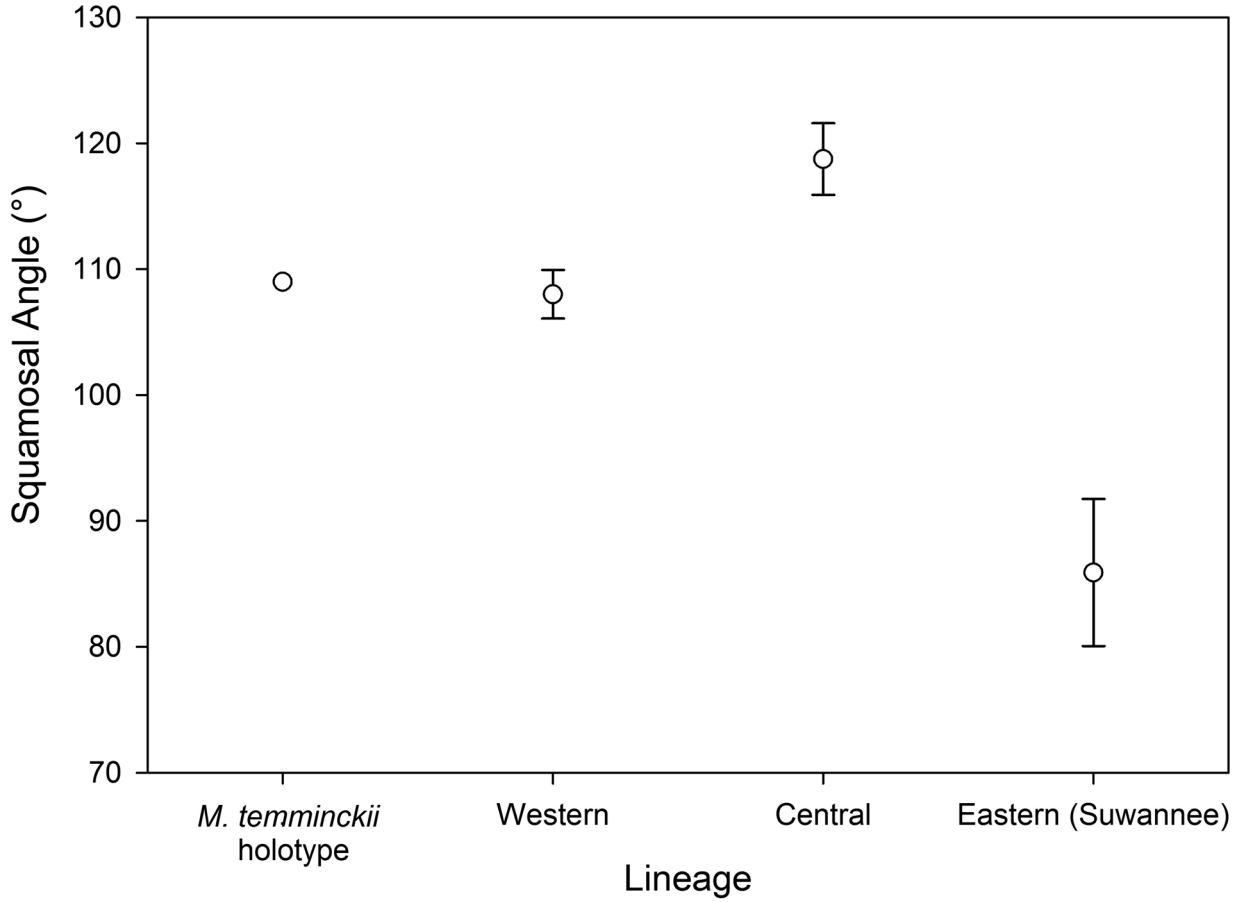


FIGURE 6. Plot of mean squamosal angle with standard error for three lineages and the holotype (MNHN-AZ-AC-A4540) of *M. temminckii.*

Species delimitation. Our GMYC results coupled with the morphological variation found among clusters suggest the presence of three separate lineages; therefore, we view these lineages as species under the General Lineage Concept of Species (de Queiroz 1998, 2007). Under this concept, secondary operational criteria (although not necessary) include that the lineage exhibits intrinsic reproductive isolation, diagnosability, and/or monophyly (de Queiroz 1998, 2007). Thus, we propose the following taxonomic revision of the genus *Macrochelys*.

Order TESTUDINES Batsch, 1788

Suborder CRYPTODIRA Cope, 1868

Family CHELYDRIDAE Swainson, 1839

***Macrochelys temminckii* (Troost *in* Harlan 1835)**

Common name. Alligator Snapping Turtle

Holotype. MNHN-AC A. 4540, "collected near Memphis" (skull also figured in Bour 1987). (Western lineage; Figure 9).

Amended diagnosis. Carapacial caudal notch narrow and triangular or U-shaped, contained wholly on the pygal and not extending onto peripheral set 11, and pygal with two serrations and without medial suture; Peripheral 11 with 1 serration; pleural scute set 1 does not overlap onto the nuchal; distal rib end of costal 1 enters middle of

peripheral 3; posterior projection of the squamosal globular and obtusely angled in lateral aspect, usually upwardly inflected; dermal scale on the frontals reduced in size; processus trochlearis oticum relatively straight with a single distal protuberance; posterior margin of squamosal-opisthotic contact relatively straight in dorsal aspect; mandible relatively narrow with slender triturating surfaces. Although generally the caudal notch is small and triangular, observable variation occurs within the species.

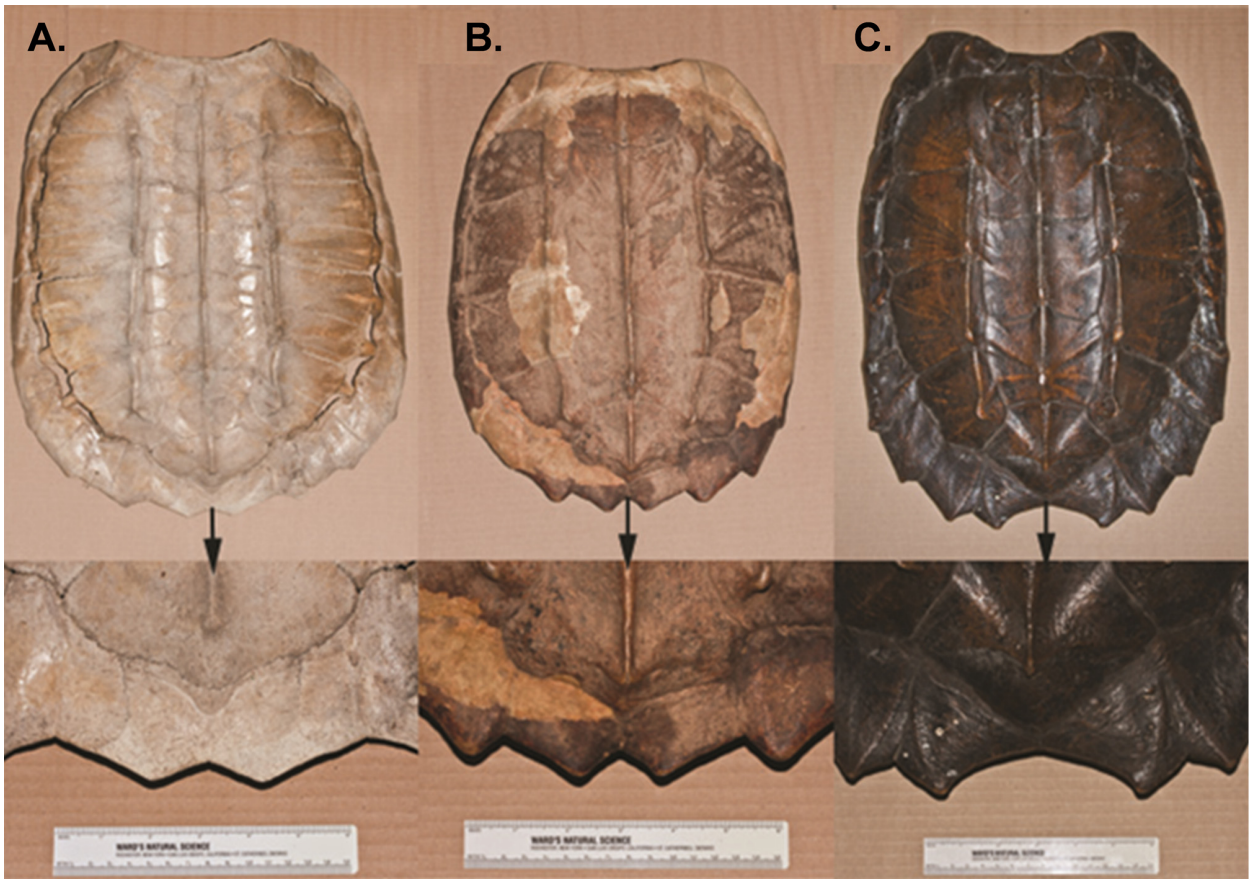


FIGURE 7. Variation of carapace morphology in western (A; UF 21746), central (B; UF 52676), and Suwannee (C; UF 57967) lineages of *Macrochelys*. Most of the gross variation in post-cranial morphology is present within the caudal region of the carapace.

Macrochelys suwanniensis sp. nov.

Common name. Suwannee Alligator Snapping Turtle

Holotype. UF 166146, adult male skeleton from Santa Fe River and State Road 235, Alachua County, Florida (29.87872ºN, 82.33619ºW, datum WGS84, elev. 23 m), found dead, apparently from gunshot wounds, in very low water in 2003 by Jason R. Bourque (see Figures 10, 11, 12). (Suwannee lineage; Figure 9).

Paratypes. UF 22267, partial skeleton from Santa Fe River, near Town of Santa Fe, Alachua County, Florida, on 9 April 1962 by George R. Zug; UF 12694, partial skeleton from Fletcher Spring, Lafayette County, Florida (29.84672ºN, 82.89256ºW, elev. 9 m), on 19 November 1961 by B. Sites, D. Desautels, and D. Young.

Diagnosis. *Macrochelys suwanniensis* is distinguished by the following: carapacial caudal notch very wide and lunate (Figure 10), usually comprising the pygal and peripheral set 11 (shared with *Chelydra*); pygal sutured medially (composed of two bones) often with no serrations; Peripheral 11 with 1–2 serrations; distal rib end of costal 1 enters posterior third of peripheral 3; pleural scute set 1 with broad overlap onto the nuchal; dermal scale on the frontals very wide; processus trochlearis oticum with developed proximal and distal protuberances; squamosal contacts opisthotic anteriorly when viewed in dorsal aspect; mandible broad with expanded triturating surfaces and developed labial rugosity just anterior to the coronoid; posterior projection of the squamosal acutely angled in lateral aspect, dorsally straight or downwardly directed, and posteriorly extensive past the plane of the quadrate (Figure 11).

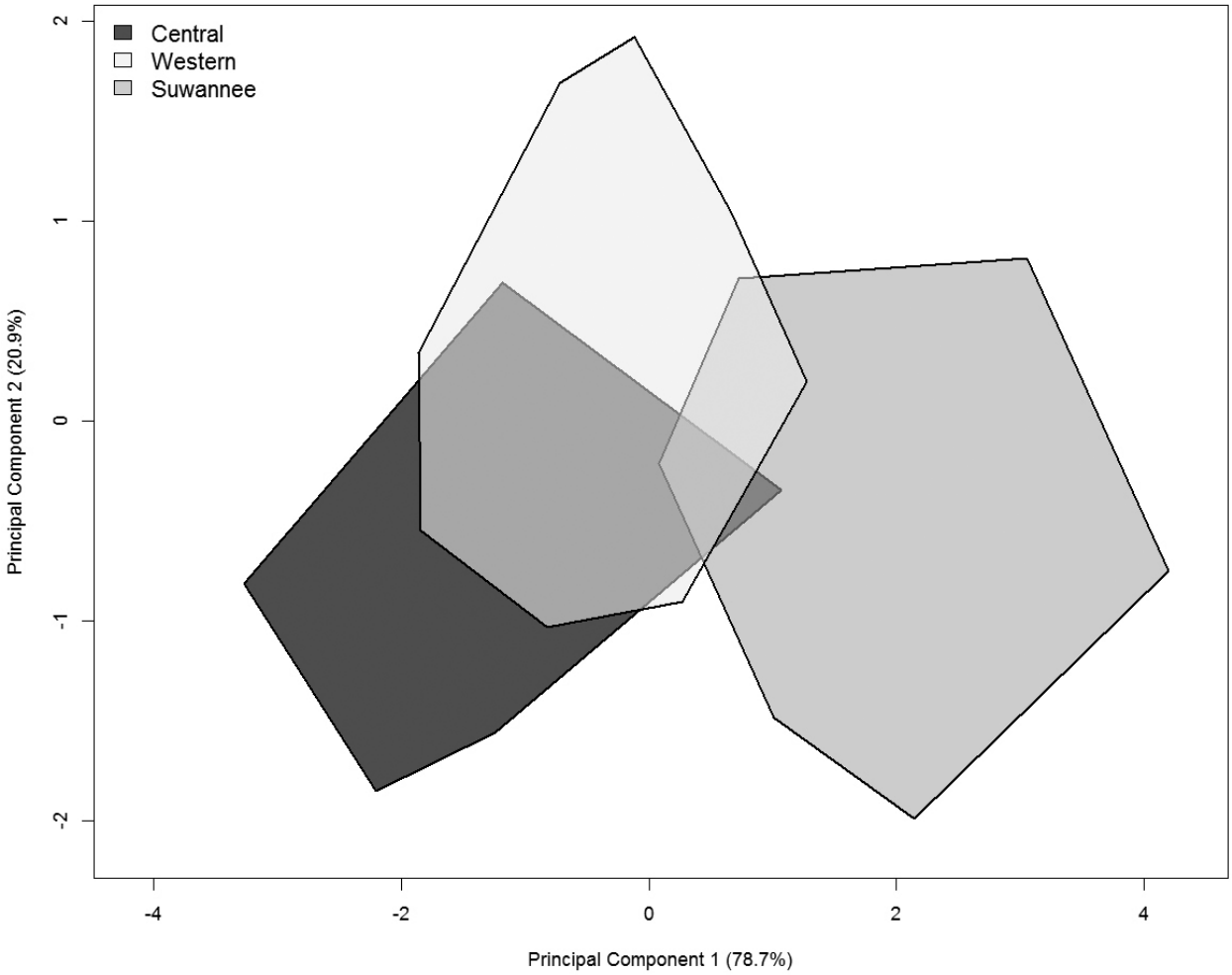


FIGURE 8. Polygons showing the principal component scores (and associated percent of variability explained by each component) for carapace morphometric measurements (caudal notch width, caudal notch area, caudal notch depth) from 104 alligator snapping turtles by lineage (Suwannee n=28, central n=15, and western n=61).

Comments. Most carapaces of *Macrochelys suwanniensis* exhibited a medially sutured pygal. This feature is significant when considering caudal notch width and is likely at least part of the reason this species possesses the widest caudal notch amongst congeners. The extra suture may allow the caudal notch to expand as the turtle grows larger. This is in contrast to *M. temminckii*, which possesses a single unsutured pygal bone and consequently the narrowest caudal notch of extant *Macrochelys*. Peripheral 11 is usually doubly serrated; i.e., the serrations that are typically contained on the pygal bone in the western and central species have migrated onto the 11th peripheral set in *M. suwanniensis*.

Distribution. Restricted to the Suwannee River drainage in Florida and Georgia.

Etymology. Specific epithet refers to combination of the new Latin *suwanni–* (referring to the Suwannee River) and the Latin *–ensis* (belongs to the) to form the composite noun *suwanniensis*.

Specimens examined. See Appendix.

Macrochelys apalachicolae sp. nov.

Common name. Apalachicola Alligator Snapping Turtle

Holotype. UF 3998, partial skeleton from the Apalachicola River, Gadsden County, Florida, on 4 April 1953 by the Florida Museum of Natural History (see Figures 13, 14). (Central lineage; Figure 9).

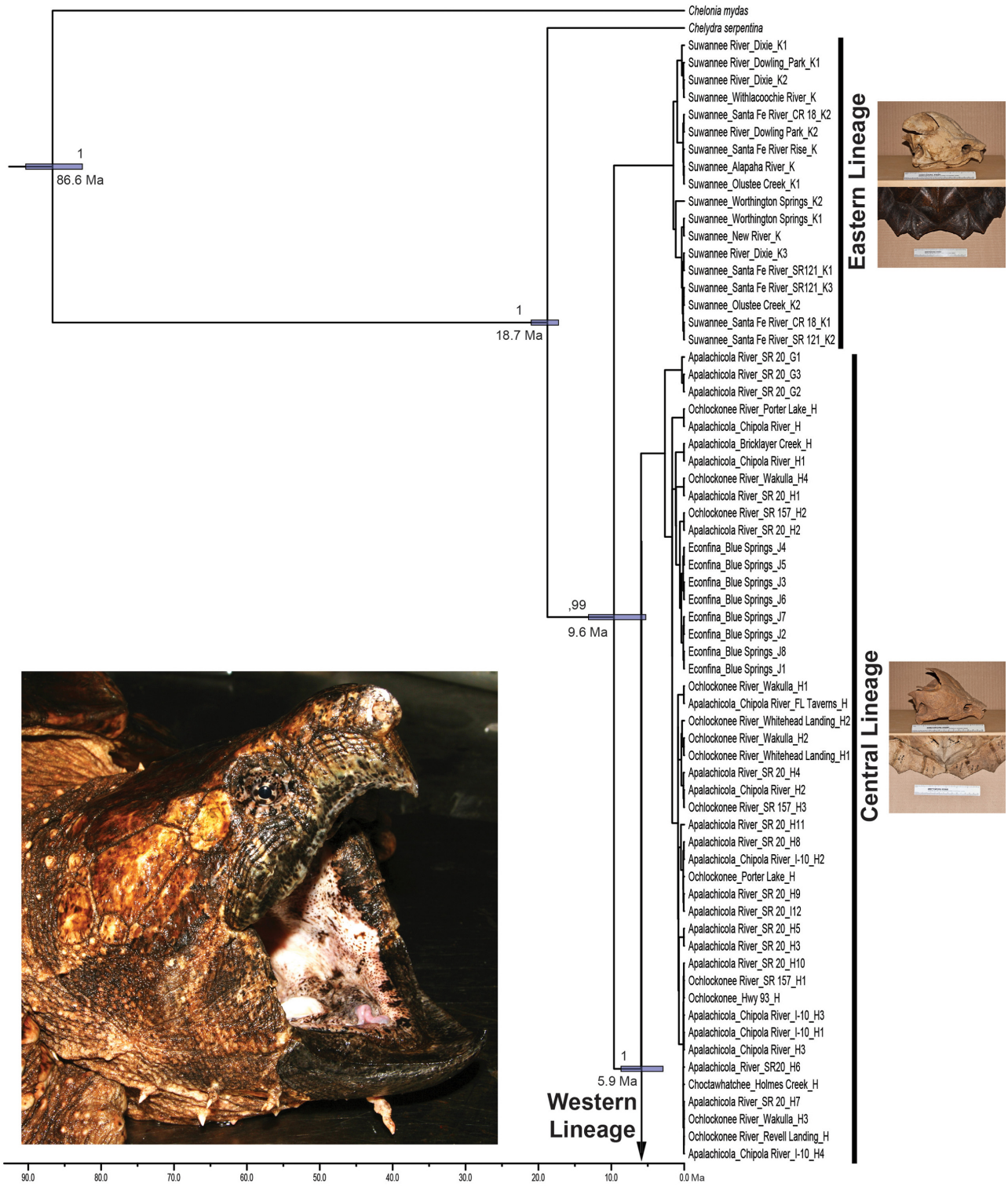


FIGURE 9. Bayesian inference phylogeny for extant chelydrids (*Chelydra* and *Macrochelys*). Note that representative skeletal synapomorphies in skull and carapace (above and below, respectively, next to lineage name) are provided for each lineage of *Macrochelys*; values above major nodes represent posterior probabilities (≥ 95%); values below major nodes represent the mean divergence time estimation of the most recent common ancestor (MRCA); and bars at major nodes represent 95% Highest Posterior Density (HPD).

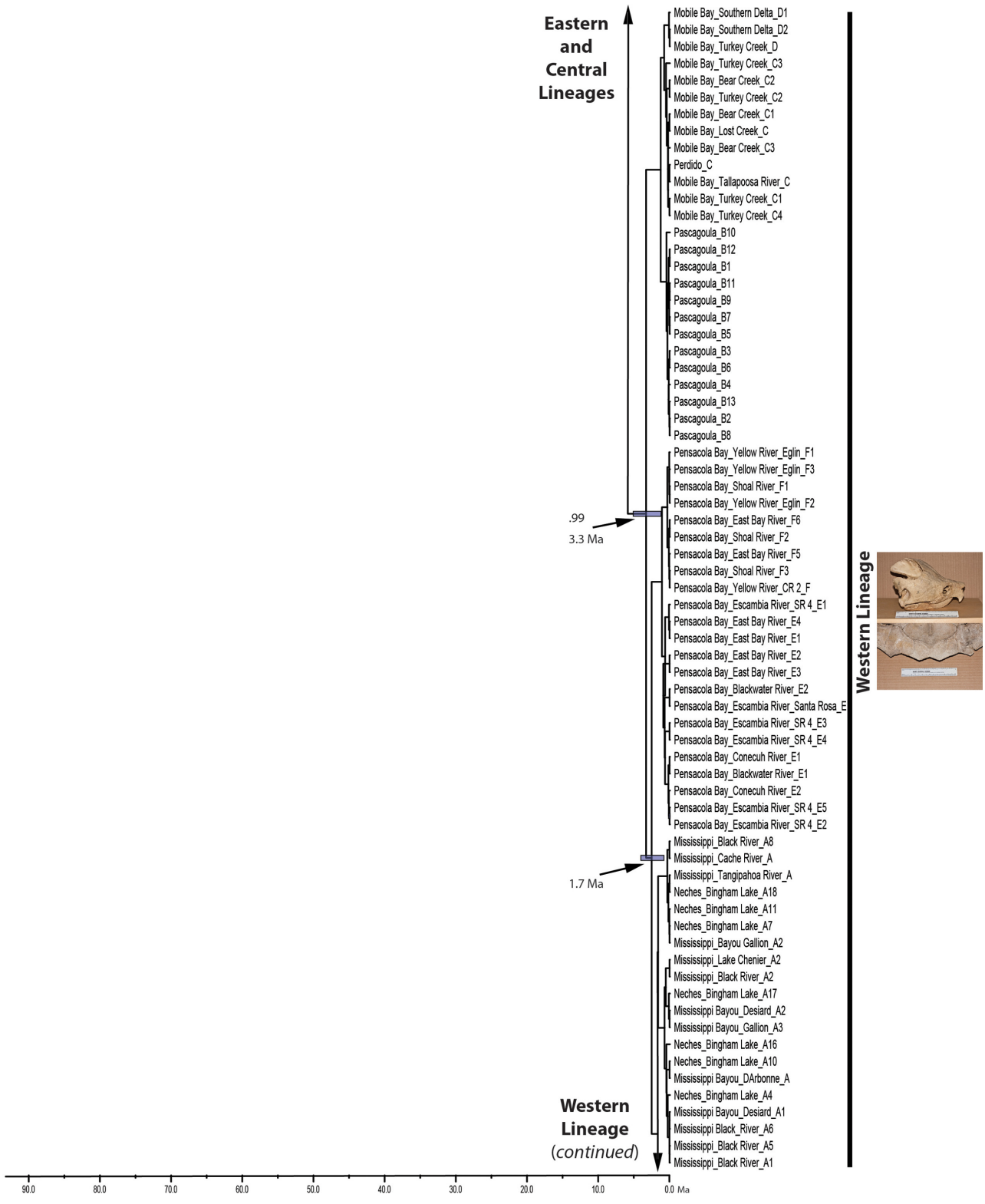


FIGURE 9. (Continued)

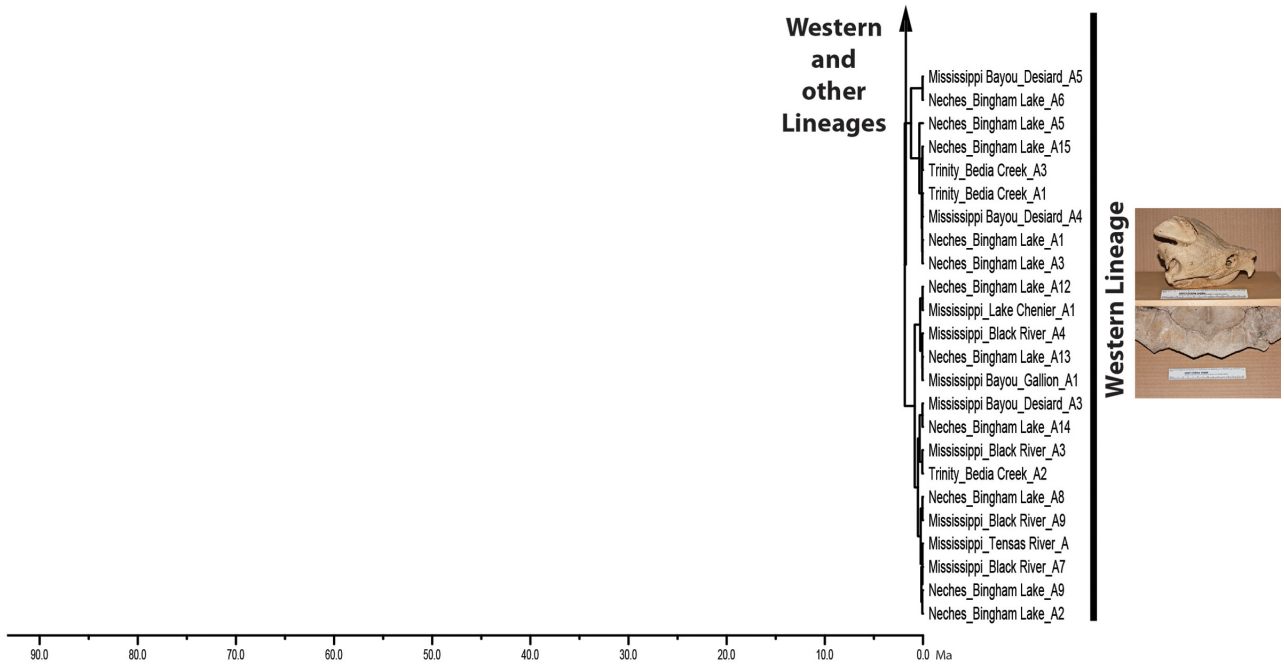


FIGURE 9. (Continued)

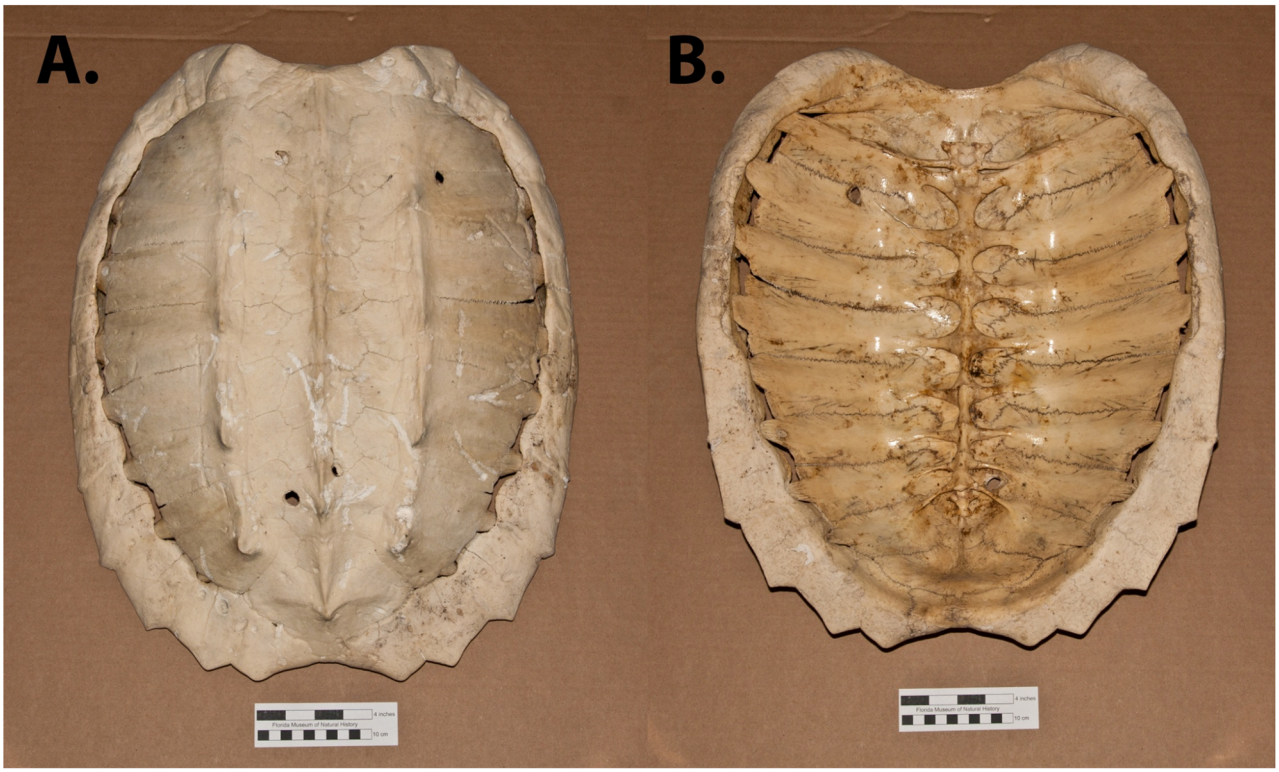


FIGURE 10. Photograph of *Macrochelys suwanniensis* holotype (UF 166146) demonstrating external (A) and internal (B) carapace morphology.

FIGURE 11. Photograph of *Macrochelys suwanniensis* holotype (UF 166146) demonstrating a superior view of plastron morphology.

Paratypes. UF 52676, partial skeleton from Waddells Mill Creek, Jackson County, Florida, on 10 April 1978 by L. Richard Franz *et al*.; UF 152479 skull from Econfina Creek, Bay County, Florida (30.15274ºN, 85.55748ºW, elev. 2 m, 13.1 m depth), on 21 August 1982 by Joseph P. Ward and Joseph J. Ward.

Diagnosis. *Macrochelys apalachicolae* is distinguished by the following: carapacial caudal notch narrow and triangular or narrow and U-shaped (Figure 13), relatively shallow, and reduced; posterior projection of the squamosal globular and obtusely angled in lateral aspect (Figure 5,14); pygal with two serrations, with medial suture; peripheral 11 with one serration; distal rib end of costal 1 enters posterior third of peripheral 3; pleural scute set 1 with slight to no overlap onto the nuchal; processus trochlearis oticum relatively straight with a single distal protuberance; posterior margin of squamosal-opisthotic contact relatively straight in dorsal aspect.

Comments. Although there is a general pattern of small triangular pygal regions of the carapace, there is observable variation within the species. All cranial specimens are characterized by large, globular squamosal projections that are intermediate between those of *M. suwannensis* and *M. temminckii*. Although *M. apalachicolae* is genetically most similar to *M. temminckii*, in some ways it is morphologically more similar to *M. suwanniensis*; they share the unique synapomorphy of a sutured pygal. *Macrochelys apalachicolae* is somewhat morphologically

intermediate between *M. temminckii* and *M. suwannensis* with regard to carapacial caudal notch proportions. The degree of overlap of pleural 1 onto the nuchal also suggests this (usually lying on or just anterior to the nuchal-costal 1 suture), as does a pygal that possesses two serrations (a western character) that is typically sutured medially (a character found in *M. suwannensis*).

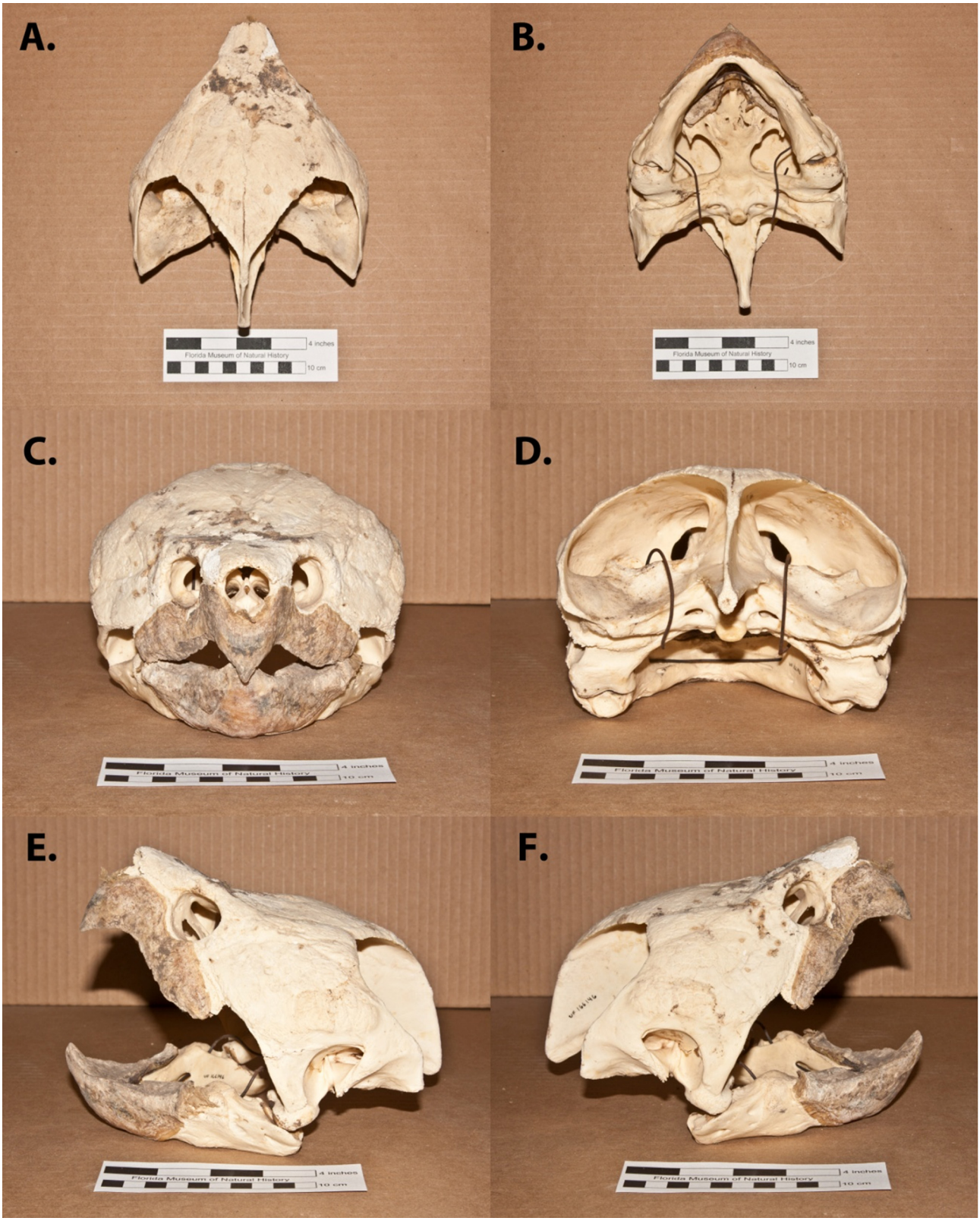


FIGURE 12. Photograph of *Macrochelys suwanniensis* holotype (UF 166146) demonstrating a superior (A), inferior (B), cranial (C), caudal (D), and left (E) and right (F) lateral view of skull morphology.

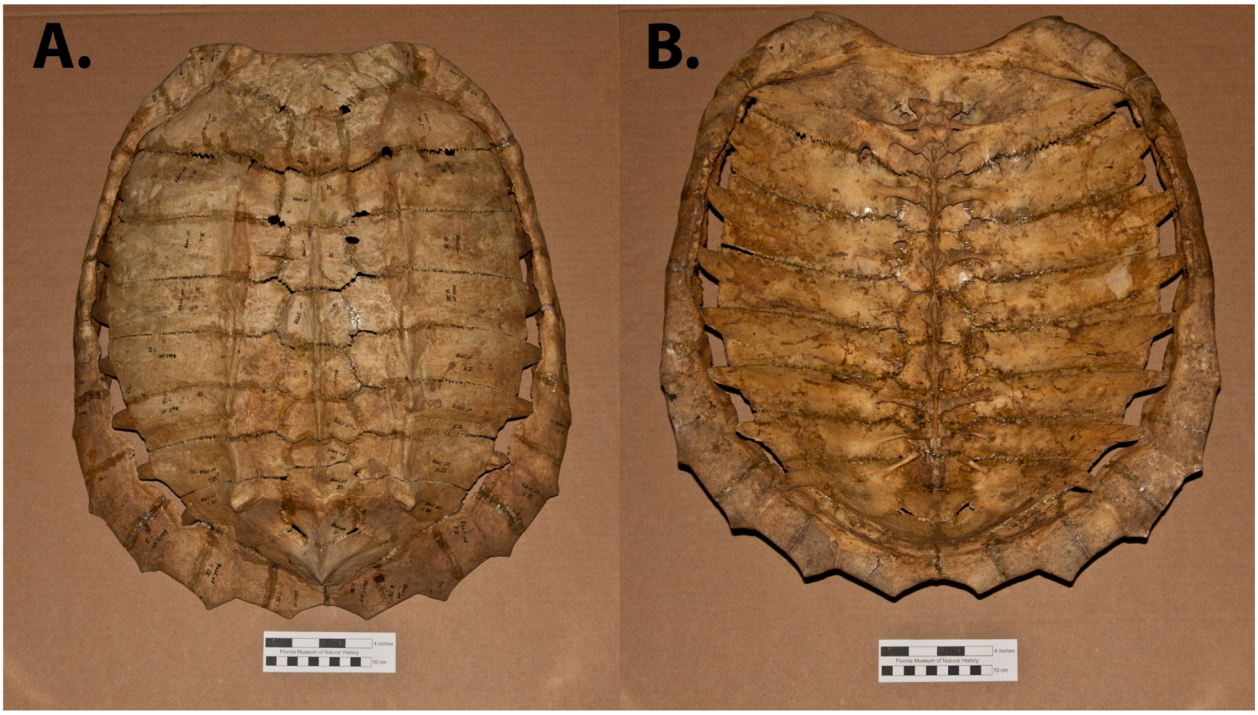


FIGURE 13. Photograph of *Macrochelys apalachicolae* holotype (UF 3998) demonstrating external (A) and internal (B) carapace morphology.

Distribution. Restricted to river drainages bounded by the Choctawhatchee and Ochlockonee rivers in Florida, Georgia, and Alabama.

Etymology. Specific epithet refers to the new Latin *apalachicol–* (referring to the Apalachicola River) and the Latin *–ae* (treating the name of the river as a Latin cognate in the First Declension, genitive case), combined to form the composite noun *apalachicolae*.

Fossil record. The earliest fossil representatives of *Macrochelys* in Florida are from the early Miocene, early Barstovian NALMA, ca. 15–16 Ma. These fossils are fragmentary and consist of a partial costal 8 (UF-Vertebrate Paleontology [VP] 259076) and partial hyo- and hypoplastron (UF-VP 259077). Although difficult to ascribe to the species level, they are contemporaneous with *Macrochelys stricta* (Matthew 1924) from the early Barstovian of Nebraska.

Additional *Macrochelys* specimens are not observed in Florida until the late Miocene, early Hemphillian NALMA, ca. 8–9 Ma, with the occurrence of *Macrochelys auffenbergi* (Dobie 1968), which is represented by fairly complete material from the McGehee Farm locality in Alachua County. We reexamined these type specimens, as well as previously undescribed specimens of *M. auffenbergi*, to diagnose the species based on shell and skull characters and to distinguish it from extant *Macrochelys*. In *M. auffenbergi*, the nuchal and cervical are relatively narrow; pleural 1 does not contact the nuchal (shared with western *M. temminckii* and *Chelydra*); pygal is much longer than wide, with two serrations and a very narrow caudal notch; pygal lacks medial suturing and is keeled along vertebral 5; epiplastra are relatively wide and lobate (these are long and slender in extant *Macrochelys*); and sulcal impressions for scutes on the plastron are distinct and deeply incised (these scales are very thin and their impressions faint to lacking in extant *Macrochelys*). In extant *Macrochelys*, the nuchal and cervical are wide; Pleural 1 does (in both *M. apalachicolae* and *M. suwanniensis*) and does not (in *M. temminckii*) contact the nuchal; the pygal is much wider than long (in both *M. apalachicolae* and *M. suwanniensis*); the epiplastra are very narrow; and the plastron lacks well-defined scute sulcal impressions. The skull of *M. auffenbergi*, although relatively large, does not exhibit the extraordinary megacephaly expressed in extant *Macrochelys*. The relative head size is much smaller in *M. auffenbergi* than in modern *Macrochelys*, and in that way the fossil taxon is plesiomorphic. The triturating surfaces of the skull and mandible are slender and not as expanded as seen in extant *Macrochelys*, perhaps an indication that the fossil taxon was less durophagous (eating

fewer hard-shelled organisms) than the extant *Macrochelys*. Increase in head size through time appears to correlate with a decrease in plastral forelobe width within *Macrochelys*.

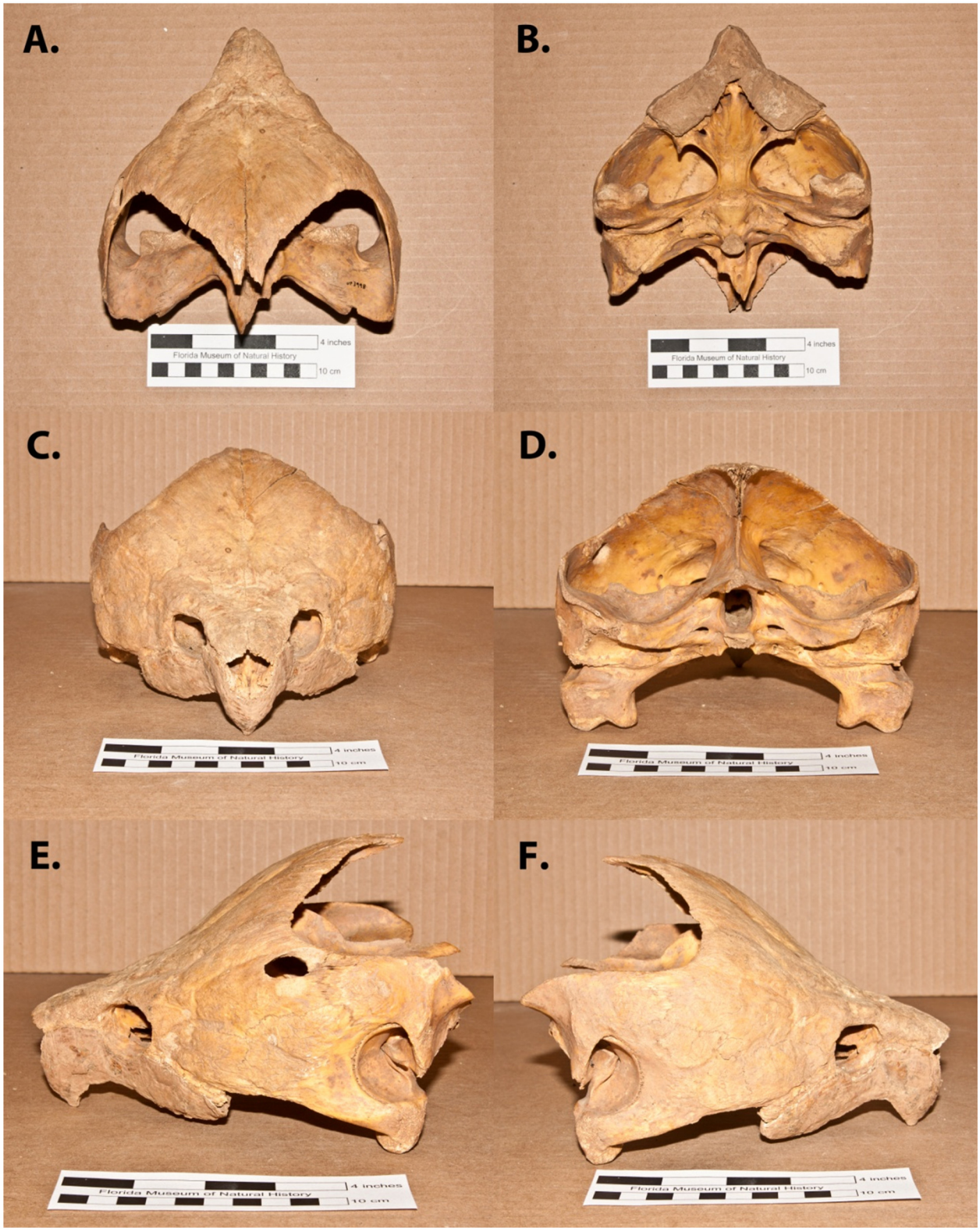
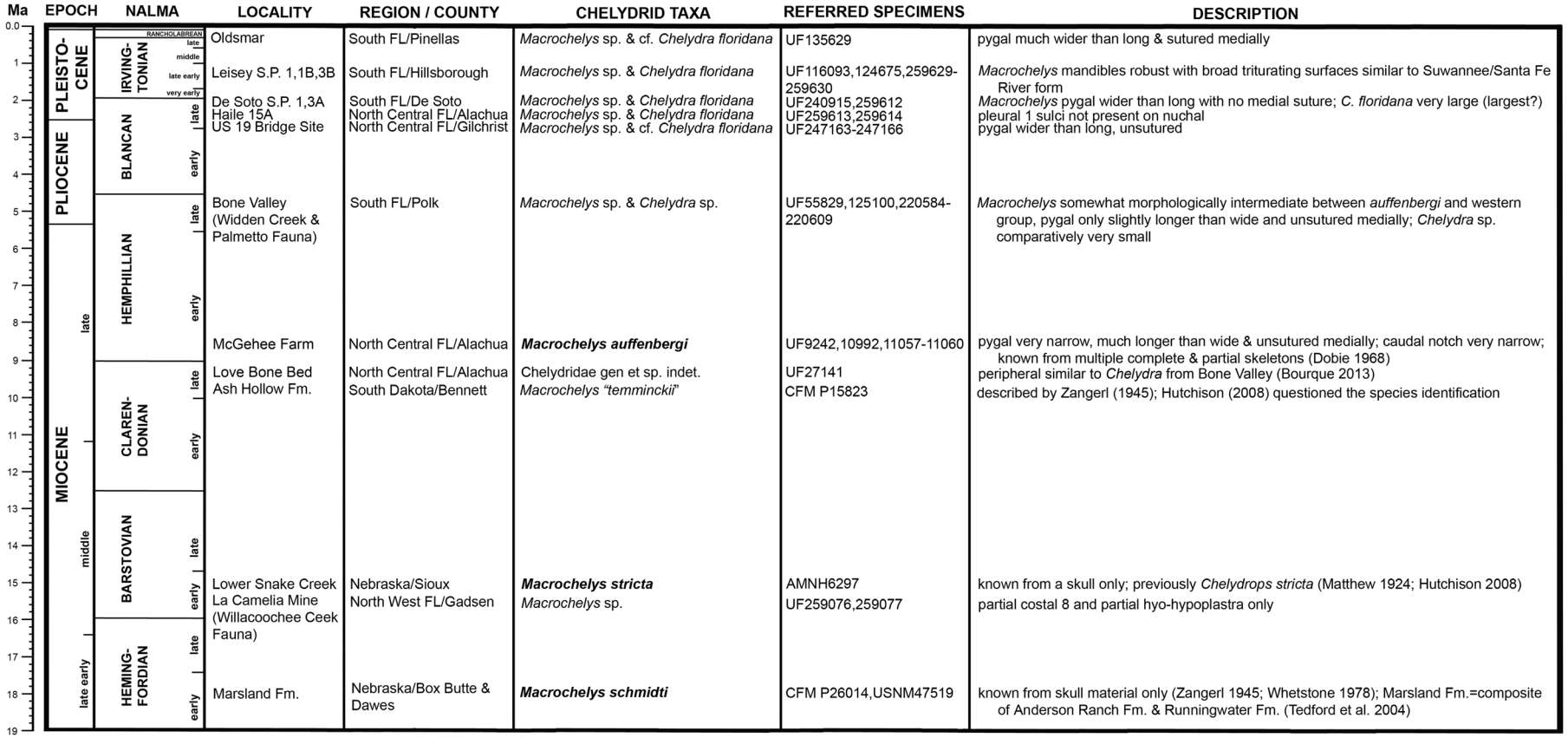


FIGURE 14. Photograph of *Macrochelys apalachicolae* holotype (UF 3998) demonstrating a superior (A), inferior (B), cranial (C), caudal (D), and left (E) and right (F) lateral view of skull morphology.



LOCALITY	REGION / COUNTY	CHELYDRID TAXA	REFERRED SPECIMENS	DESCRIPTION
Oldsmar	South FL/Pinellas	*Macrochelys* sp. & cf. *Chelydra floridana*	UF135629	pygal much wider than long & sutured medially
Leisey S.P. 1,1B,3B	South FL/Hillsborough	*Macrochelys* sp. & *Chelydra floridana*	UF116093,124675,259629-259630	*Macrochelys* mandibles robust with broad triturating surfaces similar to Suwannee/Santa Fe River form
De Soto S.P. 1,3A	South FL/De Soto	*Macrochelys* sp. & *Chelydra floridana*	UF240915,259612	*Macrochelys* pygal wider than long with no medial suture; *C. floridana* very large (largest?)
Haile 15A	North Central FL/Alachua	*Macrochelys* sp. & *Chelydra floridana*	UF259613,259614	pleural 1 sulci not present on nuchal
US 19 Bridge Site	North Central FL/Gilchrist	*Macrochelys* sp. & cf. *Chelydra floridana*	UF247163-247166	pygal wider than long, unsutured
Bone Valley (Widden Creek & Palmetto Fauna)	South FL/Polk	*Macrochelys* sp. & *Chelydra* sp.	UF55829,125100,220584-220609	*Macrochelys* somewhat morphologically intermediate between *auffenbergi* and western group, pygal only slightly longer than wide and unsutured medially; *Chelydra* sp. comparatively very small
McGehee Farm	North Central FL/Alachua	***Macrochelys auffenbergi***	UF9242,10992,11057-11060	pygal very narrow, much longer than wide & unsutured medially; caudal notch very narrow; known from multiple complete & partial skeletons (Dobie 1968)
Love Bone Bed	North Central FL/Alachua	Chelydridae gen et sp. indet.	UF27141	peripheral similar to *Chelydra* from Bone Valley (Bourque 2013)
Ash Hollow Fm.	South Dakota/Bennett	*Macrochelys "temminckii"*	CFM P15823	described by Zangerl (1945); Hutchison (2008) questioned the species identification
Lower Snake Creek	Nebraska/Sioux	***Macrochelys stricta***	AMNH6297	known from a skull only; previously *Chelydrops stricta* (Matthew 1924; Hutchison 2008)
La Camelia Mine (Willacoochee Ceek Fauna)	North West FL/Gadsen	*Macrochelys* sp.	UF259076,259077	partial costal 8 and partial hyo-hypoplastra only
Marsland Fm.	Nebraska/Box Butte & Dawes	***Macrochelys schmidti***	CFM P26014,USNM47519	known from skull material only (Zangerl 1945; Whetstone 1978); Marsland Fm.=composite of Anderson Ranch Fm. & Runningwater Fm. (Tedford et al. 2004)

FIGURE 15. Paleontological timeline of published and previously unpublished *Macrochelys* fossil specimens summarizing their associated collection locality, geological age, and identifying characteristics.

Macrochelys next appears in Florida from Polk County, from the Widden Creek and Palmetto faunas of the Bone Valley Formation, late Hemphillian, latest Miocene-earliest Pliocene (Meylan 1995). These fossils are fragmentary and occur well south of the current range of *Macrochelys*. The isolated fossil elements are difficult to identify at the species level; however, the Bone Valley taxon appears larger than *M. auffenbergi* and more comparable in size to extant specimens. A few features indicate that the fossil taxon is somewhat intermediate in morphology between *M. temminckii* and *M. auffenbergi*. These features include having a pygal that is only slightly longer than wide or almost as wide as long (in *M. auffenbergi*, the pygal is much longer than wide, and in the extant clade, it is much wider than long), and relatively long slender dentaries without overlying expanded triturating surfaces (also seen in *M. temminckii* and *M. auffenbergi*).

Macrochelys fossils are relatively common in late Blancan to Recent (from ca. 2.5 Ma) fluvial and estuarine deposits in Florida. Most of these fossils are fragmentary. Records include:

1) Late Blancan (ca. 2.5 Ma) US 19 bridge site from the Suwannee River, Gilchrist County. A pygal (UF-VP 247166) from this locality is wider than long with two serrations and unsutured medially. The dentaries (UF-VP 247163–247165) are generally slender as in *M. auffenbergi* and *M. temminckii*.

2) Late Blancan (ca. 2.5 Ma) Haile 15A locality, Alachua County. A nuchal (UF-VP 259613) possesses no Pleural 1 sulci dorsally, the same condition as in *M. auffenbergi* and *M. temminckii*.

3) Latest Blancan (ca. 2 Ma) De Soto Shell Pit locality (pits 1 and 3A), De Soto County. Records from this locality are farther south than the current range of the genus. A pygal (UF-VP 240915) from De Soto 3A is wider than long with two serrations and unsutured medially.

4) Early Irvingtonian (ca. 1.6-1.0 Ma) (Morgan & Hulbert 1995; Meylan 1995) Leisey Shell Pits (sites 1, 1B, and 3B), Hillsborough County. Both *Macrochelys* and *Chelydra* occur at this locality, and we feel that there is some confusion with regard to Meylan's (1995) *Macrochelys* vouchers. Some of the vouchers represent other taxa (e.g., UF-VP 84005 from pit 1A is half of an emydid bridge peripheral), including a giant *Chelydra* species (e.g., a partial peripheral UF-VP 81198 from pit 1A and a partial shell UF-VP 125099 from pit 2). Meylan (1995:285) regarded some of his chelydrid identifications as tentative, being aware of an unnamed contemporaneous giant *Chelydra* (*see* below for discussion of *Chelydra* species). Also, the *Macrochelys* left dentary (UF-VP 116093) reported from pit 3A is actually from pit 3B. The *Macrochelys* fossils occur south of the extant range and are significant in that the dentaries are very robust with expansive triturating surfaces like those in modern *M. suwanniensis*.

5) Latest Irvingtonian to earliest Rancholabrean (ca. 0.3 Ma) Oldsmar locality, Pinellas County (Meylan 1995). UF-VP 135629 represents a partial posterior carapace with the pygal region well preserved. As with the Leisey specimens, this specimen most closely resembles *M. apalachicolae* and *M. suwanniensis*. The pygal is much wider than long, is sutured medially, and possesses two serrations, the condition most typically seen in the central assemblage.

6) Numerous late Pleistocene Rancholabrean NALMA records exist from Florida, including: Jug Springs, Ichetucknee River, Columbia County (Auffenberg 1957); Suwannee River sites; Hornsby Springs, Santa Fe River, Alachua County; and Aucilla River 1A, Taylor County; as well as extralimital occurences from Wekiva Spring, Levy County; Rock Springs, Orange County; Oklawaha 1, Oklawaha River, Marion County; and Buzzard Island, Putnam County (Meylan 1995). Most of the Rancholabrean fossils are difficult to assess at the species level due to their incompleteness. Specimens from the Ichetucknee River (Suwannee River drainage), including some previously discussed by Auffenberg (1957), consist of nearly complete shells and skulls (e.g., UF-VP 259848, UF-VP 259849, and UF-VP 259842). However, the squamosal and pygal regions are not preserved in these fossils. In UF-VP 259848 and UF-VP 259849, peripheral 11 possesses only one serration, indicating the pygal was also serrated.

Discussion

Each of the three genetically distinct *Macrochelys* lineages can be diagnosed morphologically, and these differences, at least within *M. suwanniensis*, can be observed by gross visual inspection (Figure 5, 7). Although there is individual variation, morphological distinctiveness of each assemblage can be detected by simple linear

measurements and image analysis and/or gross visual inspection. *Macrochelys suwanniensis* is the most morphologically distinctive; the carapace can usually be differentiated by the presence of a large, lunate caudal notch. Conversely, *M. temminckii* and *M. apalachicolae* have narrow, triangular or U-shaped caudal notches that, although statistically different, are more difficult to differentiate from each other. A similar pattern appears in the skull, with both *M. temminckii* and *M. apalachicolae* displaying large, globular squamosal projections, whereas *M. suwanniensis* has an acute, sharp squamosal projection (Figure 5). The Suwannee River was created by the formation of the Okefenokee Swamp during the late Neogene to Quaternary (Carver *et al*. 1986). Seven rivers (Steinhatchee, Fenholloway, Econfina, Aucilla, Wacissa, Saint Marks, and Wakulla) between the Suwannee and Ochlockonee rivers lack vouchered specimens (Ewert *et al*. 2006), and this apparent distributional gap and subsequent geographic isolation have likely resulted in *M. suwanniensis* being the most genetically and morphologically distinct of the three *Macrochelys* lineages.

Although Hutchison (2008) noted that *Chelydra* and *Macrochelys* likely diverged by the late Eocene (ca. 39 Ma, late Duchesnean), the fossils on which this date is based are taxonomically uncertain and, thus, were not used as the calibration point for divergence between these two genera in this study. Roman *et al*. (1999) used an arbitrary molecular clock of up to 1.2–2.4% per million years and suggested that the three extant *Macrochelys* lineages diverged sometime between the Pliocene to early Pleistocene. Because strict molecular clock estimates are often arbitrary and unrealistic (Drummond *et al*. 2006), our relaxed clock divergence estimates are earlier than those previously reported (Roman 1999). Divergences among *Macrochelys* samples within each of the three lineages occurred less than 3.5 Ma (Figure 9), and our estimates of exponential growth rate (*g*) for *M. temminckii* and *M. apalachicolae* suggest stable population sizes over time. However, Echelle *et al*. (2010) used microsatellite data to suggest past population bottlenecks.

With regards to *Macrochelys* taxonomy, one must consider the available name for the fossil species *Macrochelys floridana*. Hay (1907, 1908) described *M. floridana* from the Pleistocene of Hillsborough County, Florida, but Auffenberg (1957) later synonymized it with *Macrochelys temminckii*. Hay referred to four specimens of *M. floridana*, but unfortunately he designated no holotype. Upon reexamination of *M. floridana* specimens, we feel that most or all of these probably represent the genus *Chelydra*. The co-occurrence of *Macrochelys* and *Chelydra* is common in late Blancan through Irvingtonian deposits in Florida (see Figure 15), and it would not be unusual if Hay's (1907, 1908) fossils represented both genera. None of Hay's fossils are diagnostic at the species level with regards to the genus *Macrochelys*. However, USNM 16674–16677 are slightly more diagnostic with regards to *Chelydra* and probably represent a giant extinct species from the early Pleistocene of Florida. More complete specimens of this giant *Chelydra* have been referred to in more recent literature from the Blancan NALMA sites Haile 7C and Haile 15A of Alachua County, Florida, as *Chelydra* sp. nov. (Morgan & Hulbert 1995:68–69). We recommend the name *Chelydra floridana* be utilized in future descriptions of this giant *Chelydra* and that USNM 16676 (Hay 1907, 1908) be designated the lectotype because it is the most diagnostic of Hay's referred fossils and clearly represents a very large *Chelydra*. In USNM 16676, the marginal-pleural sulcus is located distally from the peripheral-costal suture and the peripheral lacks inframarginal scutes. These features are characteristic of *Chelydra*, while in *Macrochelys*, the marginal-pleural sulcus would be positioned more proximally to the peripheral-costal suture and inframarginal scute sulci would typically be present on the dorsal face of the bone.

Hoser (2013) attempted to describe a new species, *Macrochelys maxhoseri*, and subspecies, *M. temmincki* (sic) *muscati*, in his self–published, non peer-reviewed "journal," but he erred in his methods. In designating holotypes using an online database in lieu of actually examining specimens, Hoser declared "specimens" UF 155266 and UF 165801 as primary types. However, the curator of herpetology at the FLMNH indicated that physical specimens bearing either of these numbers have never existed among their holdings; the corresponding records in the FLMNH database refer to unvouchered field sightings of *Macrochelys* (M.A. Nickerson, Pers. Comm. 2013). Hoser's holotypes are therefore designated in violation of ICZN Code Article 16.4 (they are not based on specimens; ICZN, 1999), and his names for *Macrochelys* are rendered unavailable.

Conservation implications. Some *Macrochelys* populations were sharply reduced by commercial harvest for the turtle meat and soup market in the 1970s and 1980s (Sloan & Lovich 1995; Pritchard 2006). In addition to high harvest rates, the long-term persistence of many *Macrochelys* populations has been a concern because of biological attributes such as long life span and low reproductive rates (Tucker & Sloan 1997; Reed *et al*. 2002). *Macrochelys temminckii* (sensu lato) was listed in 2006 in Appendix III of the Convention on International Trade in Endangered

Species (CITES), which strengthened regulations related to international trade. The species-level breaks found in this study indicate that *Macrochelys* should be managed as three separate species, with *Macrochelys suwanniensis* being restricted to Florida and Georgia and *M. apalachicolae* being restricted to Florida, Georgia, and Alabama. Management of *Macrochelys* continues largely at the state level, and our taxonomic revision may necessitate review of current state management strategies.

In Florida, *M. temminckii* is presently listed as a Species of Special Concern, but in 2010, the Florida Fish and Wildlife Conservation Commission (FWC) reviewed the status of this as a single species. The FWC, following the protocols of the IUCN Red List Criteria at Regional Levels (Version 3.0) and guidelines for Using the IUCN Red List Categories and Criteria (Version 8.1), determined that *M. temminckii* did not meet the criteria for listing, and it is scheduled to be removed from the Species of Special Concern list once a management plan is approved (Florida Fish and Wildlife Conservation Commission 2011). With the recognition of *M. temminckii*, *M. apalachicolae,* and *M. suwanniensis*, Florida will be the only state to possess all three species, and it will be necessary to review the status of each species independently. Regardless of the outcome of such reviews, *Macrochelys* will still be protected by regulations that prohibit take and possession in Florida.

Presently, Georgia lists *Macrochelys* as Threatened under its Endangered Wildlife Act of 1973 (391-4-10-.08). A study conducted in Georgia in 1997–2001 showed great variation in relative density among drainages. High capture rates of *M. apalachicolae* were found in the Apalachicola River drainage (Jensen & Birkhead 2003); however, a previous survey conducted in the Flint River (part of the Apalachicola drainage) showed much lower capture rates (Johnson 1989). In the past, *M. apalachicolae* was heavily harvested within the Flint River system. One trapper, Al Redmond, was thought to have harvested 4000–5000 adult *M. apalachicolae* during 1971–1983 (Johnson 1989). Although a few *M. suwanniensis* were captured in tributaries of the Suwannee River (Alapaha, Little, and Withlacoochee rivers), this species was not found in the Suwannee River within Georgia despite intensive surveys (Jensen & Birkhead 2003). It appears that the distribution of *M. suwanniensis* may be extremely limited within Georgia; thus, further surveys and protection efforts throughout the Suwannee River drainage are warranted.

Alabama lists *M. temminckii* as Threatened, and it is illegal to take, possess, capture, sell, or trade without a permit (Ala.Admin.Code r. 220-2-.92 [1990]). Although *M. temminckii* is found throughout much of the state, *M. apalachicolae* is only present within two river systems in Alabama: the Chattahoochee River and its tributaries (Apalachicola River drainage) and the Choctawhatchee River drainage (Choctawhatchee River, Little Choctawhatchee River, and Pea River). The distribution of *M. apalachicolae* within these two river drainages is unknown, and further surveys are necessary to examine its conservation status in the state.

Many threats persist for all three species of *Macrochelys*. Despite widespread protections against harvest, concerns about illegal take remain. Accidental ingestion of fishing hooks can perforate the digestive tract lining, and the associated monofilament, twine, or gel spun fishing line can cause plication of the small and large intestine with potential rupture resulting in injury or death (D. J. Heard, pers. Comm. 2013). A threat to the genetic integrity of populations is the release of pet-trade turtles. *Macrochelys* continues to be widely available in the pet trade. Internet sales and exotic pet shows in states without restrictions on possession make it difficult to stop the importation of pet-trade animals into states like Florida, Georgia, and Alabama that have possession restrictions. Introduced and translocated animals have the potential to disrupt barriers to dispersal that have persisted for millions of years. For example, releases of pet turtles are presumably responsible for the capture of *Macrochelys* in Florida in river systems or counties outside of their known range: Marion County (AMNH 8287, KU 61844); Duval County; Alafia River, Hillsborough County; St. Johns River, Orange County; and Hogtown Creek and Lake Wauberg, Alachua County. To protect the genetic integrity of this newly discovered species complex, restrictions are needed to prevent the introduction of extralimital animals.

Acknowledgments

Harold A. Dundee and Peter C. H. Pritchard provided access to specimens under their care at the Tulane University Museum of Natural History and the Chelonian Research Institute, respectively. Dana Ehret and Michael Brett-Surman provided photographs of *M. floridana* for examination. Salvador Bailon provided photographs of the holotype of *M. temminckii* for examination. Perran Ross provided equipment. Jerry Johnston supplemented our

dataset with additional field-captured animals. Svetlana Nikolaeva and Doug Yanega provided nomenclature recommendations. We thank Brian Bowen, F. Wayne King, John Iverson, Max Nickerson, Robert H. Robins, Savanna Barry, and Brian I. Crother for comments on a draft of this paper.

References

Aris-Brosou, S. & Yang, Z.H. (2002) Effects of models of rate evolution on estimation of divergence dates with special reference to the metazoan 18S ribosomal RNA phylogeny. *Systematic Biology*, 51, 703–714.

Auffenberg, W. (1957) The status of the turtle *Macroclemys floridana* Hay. *Herpetologica*, 13, 123–126.

Bour, R. (1987) Type-specimen of the alligator snapper, *Macroclemys temminckii* (Harlan, 1835). *Journal of Herpetology,* 21, 340–343.
http://dx.doi.org/10.2307/1563980

Bourque, J. R. 2013. Fossil Kinosternidae from the Oligocene and Miocene of Florida, USA. *In:* Brinkman, D.B., Holroyd, P.A. & Gardner, J.D. (eds.), Morphology and Evolution of Turtles. Vertebrate Paleobiology and Paleoanthropology Series, Springer, Dordrecht, Netherlands, pp. 459–475

Carver, R.E., Brook, G.A. & Hyatt, R.A. (1986) *Trail Ridge and Okefenokee Swamp*. Centennial Field Guide, Southeastern Section, 6, pp. 331–334.

Dayrat, B. (2005) Towards Integrated Taxonomy. *Biological Journal of the Linnean Society*, 85, 407–415.
http://dx.doi.org/10.1111/j.1095-8312.2005.00503.x

De Queiroz, K. & Good, D.A. (1997) Phenetic clustering in biology: A critique. *Quarterly Review of Biology*, 72, 3–30.
http://dx.doi.org/10.1086/419656

De Queiroz, K. (1998) The general lineage concept of species, species criteria, and the process of speciation: A conceptual unification and terminological recommendations. *In*: Howard, D.J. & Berlocher, S.H. (Eds.), *Endless forms*: *Species and speciation.* Oxford University Press, Inc., New York, New York, USA, pp. 57–75.

De Queiroz, K. (2007) Species concepts and species delimitation. *Systematic Biology*, 56, 879–86.

DeSalle, R., Egan, M.G. & Siddal, M. (2005) The unholy trinity: taxonomy, species delimitation, and DNA barcoding. *Philosophical Transactions of the Royal Society B*, 360, 1905–1916.
http://dx.doi.org/10.1098/rstb.2005.1722

Dobie, J.L. (1968) A new turtle species of the genus *Macrochelys* (Chelydridae) from the Florida Pliocene. *Tulane Studies in Zoology*, 15, 59–63.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. (2006) Relaxed phylogenetics and dating with confidence. *PLOS Biology*, 4, 699–710.
http://dx.doi.org/10.1371/journal.pbio.0040088

Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, 7, 214.
http://dx.doi.org/10.1186/1471-2148-7-214

Drummond, C.S., Smith, C.I. & Pellmyr, O. (2009) Species identification and sibship assignment of sympatric larvae in the yucca moths *Tegeticula synthetica* and *Tegeticula antithetica* (Lepidoptera: Prodoxidae). *Molecular Ecology Resources,* 9, 1369–1372.
http://dx.doi.org/10.1111/j.1755-0998.2009.02696.x

Echelle, A.A., Hackler, J.C., Lack, J.B., Ballard, S.R., Roman, J., Fox, S.F., Leslie, D.M. Jr. & Van Den Bussche, R.A. (2010) Conservation genetics of the alligator snapping turtle: Cytonuclear evidence of range-wide bottleneck effects and unusually pronounced geographic structure. *Conservation Genetics,* 11, 1375–1387.
http://dx.doi.org/10.1007/s10592-009-9966-1

Ezard, T.H.G. & Purvis, A. (2009) paleoPhylo: free software to draw paleobiological phylogenies. *Paleobiology*, 35, 460–464.
http://dx.doi.org/10.1666/0094-8373-35.3.460

Ewert, M.A., Jackson, D.R. & Moler, P.E. (2006) *Macrochelys temminckii* – alligator snapping turtle. *In*: Meylan, P.A. (Ed.), *Biology and Conservation of Florida Turtles*. Chelonian Research Monographs, Lunenburg, Massachusetts, pp. 58–71.

Felsenstein, J. (2004) *Inferring Phylogenies*. Sinauer Associates, Inc., Sunderland, Massachusetts, 664 pp.

Florida Fish and Wildlife Conservation Commission (2011) Alligator snapping turtle biological status review report. Florida Fish and Wildlife Conservation Commission, Tallahassee, Florida, USA, 16 pp.

Harrel, J.B., Allen, C.M. & Hebert, S.J. (1996) Movements and habitat use of subadult alligator snapping turtles (*Macroclemys temminckii*) in Louisiana. *American Midland Naturalist*, 135, 60–67.
http://dx.doi.org/10.2307/2426872

Hasegawa, M., Kishino, H. & Yano, T. (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, 22, 160–174.
http://dx.doi.org/10.1007/bf02101694

Hay, O.P. (1907) Descriptions of seven new species of turtles from the Tertiary of the United States. *Bulletin of the American Natural History Museum*, 23, 847–863.

Hay, O.P. (1908) The fossil turtles of North America. *Publications of the Carnegie Institution*, 75, 1–568.
Hillis, D.M. & Bull, J.J. (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology*, 42, 182–192.
Ho, S.Y.W. & Phillips, M.J. (2009) Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. *Systematic Biology,* 58, 367–380.
Hoser, R.T. (2013) An updated taxonomy of the living alligator snapping turtles (*Macrochelys* Gray, 1856), with descriptions of a new tribe, new species and new subspecies. *Australasian Journal of Herpetology*, 16, 53–63.
Hutchison, J.H. (2008) History of fossil Chelydridae. *In*: Steyermark, A.C., Finkler, M.S., Brooks, R.J. & Gibbons, W. (Eds.), *Biology of the snapping turtle*. Johns Hopkins University Press, Baltimore, Maryland, USA, pp. 14–30.
Jensen, J.B. & Birkhead, W.S. (2003) Distribution and status of the alligator snapping turtle (*Macrochelys temminckii*) in Georgia. *Southeastern Naturalist*, 2, 25–34.
http://dx.doi.org/10.1656/1528-7092(2003)002[0025:dasota]2.0.co;2
Johnson, S. (1989*) Population status of the alligator snapping turtle (Macroclemys temminckii) in the Flint River*. Annual performance report, Georgia Department of Natural Resouces, Forsyth, Georgia, 11 pp.
Jungers, W.L., Falsetti, A.B. & Wall, C.E. (1995) Shape, relative size and size-adjustments in morphometrics. *Yearbook of Physical Anthropology*, 38, 137–161.
http://dx.doi.org/10.1002/ajpa.1330380608
Kuhner, M.K. (2009) Coalescent genealogy samplers: Windows into population history. *Trends in Ecology & Evolution*, 24, 86–93.
http://dx.doi.org/10.1016/j.tree.2008.09.007
Kumazawa, Y. & Nishida, M. (1999) Complete mitochondrial DNA sequences of the green turtle and blue-tailed mole skink: Statistical evidence for Archosaurian affinity of turtles. *Molecular Biology and Evolution*, 16, 784–792.
http://dx.doi.org/10.1093/oxfordjournals.molbev.a026163
Lohse, K. (2009) Can mtDNA barcodes be used to delimit species? A response to Pons *et al*. (2006). *Systematic Biology*, 58, 439–442.
Matthews, W.D. (1924) Third contribution to the Snake Creek fauna. *Bulletin of American Natural Hist*ory, 50, 59–210.
Meylan, P.A. (1995) Pleistocene amphibians and reptiles from the Leisey Shell Pit, Hillsborough, County, Florida. *Bulletin of the Florida Museum of Natural History*, 37, 273–297.
Morgan, G.S. & Hulbert Jr., R.C. (1995) Overview of the geology and vertebrate biochronology of the Leisey Shell Pit local fauna, Hillsborough County, Florida. *Bulletin of the Florida Museum of Natural History,* 37, 1–92.
Morrison, D.A. (2008) How to summarize estimates of ancestral divergence times. *Evolutionary Bioinformatics*, 4, 75–95.
Near, T.J., Meylan, P.A. & Shaffer, H.B. (2005) Assessing concordance of fossil calibration points in molecular clock studies: An example using turtles. *American Naturalist,* 165, 137–146.
http://dx.doi.org/10.1086/427734
Neff, N.A. & Marcus, L.F. (1980) *A survey of multivariate methods for systematics*. Privately published, American Museum of Natural History Press, New York, USA, 243 pp.
Papadopoulou, A., Monaghan, M.T., Barraclough, T.G. & Vogler, A.P. (2009) Sampling error does not invalidate the Yule-coalescent model for species delimitation. A response to Lohse (2009). *Systematic Biology*, 58, 442–444.
Pons, J., Barraclough, T.G., Gomez-Zurita, J., Cardoso, A., Duran, D.P., Hazell, S., Kamoun, S., Sumlin, W.D. & Vogler, A.P. (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology*, 55, 595–609.
Posada, D. (2008) jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution,* 25, 1253–1256.
http://dx.doi.org/10.1093/molbev/msn083
Pritchard, P.C.H. (2006) *The Alligator Snapping Turtle*: *Biology and Conservation*. Krieger Publishing, Malabar, Florida, USA, 140 pp.
Reed, R.N., Congdon, J.D. & Gibbons, J.W. (2002) The alligator snapping turtle [*Macroclemys (Macrochelys) temminckii*]: A review of ecology, life history, and conservation, with demographic analyses of the sustainability of take from wild populations. Report to Division of Scientific Authority, U.S. Fish and Wildlife Service. 20 p. Savannah River Ecology Laboratory, University of Georgia.
Reid, N.M. & Carstens, B.C. (2012) Phylogenetic estimation error can decrease the accuracy of species delimitation: A Bayesian implementation of the General Mixed Yule Coalescent model. *BMC Evolutionary Biology*, 12 (196), 1–11.
Roman, J., Santhuff, S.D, Moler, P.E. & Bowen, B.W. (1999) Population structure and cryptic evolutionary units in the alligator snapping turtle. *Conservation Biology*, 13, 135–142.
http://dx.doi.org/10.1046/j.1523-1739.1999.98007.x
Rozas, J. (2009) DNA sequence polymorphism analysis using DnaSP. *In*: Posada, D. (Eds.), *Bioinformatics for DNA Sequence Analysis.* Humana Press Inc., Totowa, New Jersey, USA, pp. 337–350.
Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9, 671–675.
http://dx.doi.org/10.1038/nmeth.2089
Shaffer, H.B. & Thomson, R.C. (2007) Delimiting species in recent radiations. *Systematic Biology*, 56, 896–906.
Shirley, M.H., Vleit K.A., Carr, A.N. & Austin, J.D. (2014) Rigorous approaches to species delimitation have significant

implications for African crocodilian systematic and conservation. *Proceedings of the Royal Society of London B*, 281, 20132483.

Sloan, K. & Lovich, J.E. (1995) Exploitation of the alligator snapping turtle, *Macroclemys temminckii*, in Louisiana: A case study. *Chelonian Conservation and Biology,* 1, 221–222.

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

Talavera, G., Dinca, V. & Vila, R. (2013) Factors affecting species delimitations with the GMYC model: insights from a butterfly survey. *Methods in Ecology and Evolution*, 4, 1101–1110. http://dx.doi.org/10.1111/2041-210x.12107

Tedford, R.H., Albright III, L.B., Barnosky, A.D., Ferrusquia-Villafranca, I., Hunt, R.M. Jr., Storer, J.E., Swisher III, C.C., Voorhies, M.R., Webb, S.D. & Whistler, D.P. (2004) Mammalian biochronology of the Arikareean through Hemphillian interval (Late Oligocene through Early Pliocene epochs). *In*: Woodburne, M.O. (Eds.), *Late Cretaceous and Cenozoic Mammals of North America: Biostratigraphy and Geochronology*. Columbia University Press, New York, USA, pp. 169–231.

Troost, G. (1835) *Medical and Physical Researches*. Bailey, Philadelphia, 653 pp.

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

Walker, D., Moler, P.E., Buhlmann, K.A. & Avise, J.C. (1998) Phylogeographic uniformity in mitochondrial DNA of the snapping turtle (*Chelydra serpentina*). *Animal Conservation,* 1, 55–60. http://dx.doi.org/10.1111/j.1469-1795.1998.tb00226.x

Wiens, J.J. & Servedio, M.R. (2000) Species delimitation in systematic: inferring diagnostic differences between species. *Proceedings of the Royal Society of London B*, 267, 631–636. http://dx.doi.org/10.1098/rspb.2000.1049

Wiens, J.J. & Penkrot, T.L. (2002) Delimiting species based on DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). *Systematic Biology,* 51, 69–91.

Wiens, J.J. (2004) The role of morphological data in phylogeny reconstruction. *Systematic Biology*, 53, 653–661.

Wiens, J.J. (2007) Species delimitation: new approaches for discovering diversity. *Systematic Biology,* 56, 875–878.

Weinstock , J., Willerslev, E., Sher, A., Tong, W.F., Ho, S.Y.W., Rubenstein, D., Storer, J., Burns, J., Martin, L., Bravi, C., Prieto, A., Froese, D., Scott, E., Xulong, S. & Cooper, A. (2005) Evolution, systematics, and phylogeography of Pleistocene horses in the New World: A molecular perspective. *PLOS Biology,* 3, 1373–1379. http://dx.doi.org/10.1371/journal.pbio.0030241

Zuckerkandl, E. & Pauling, L. (1965) Evolutionary divergence and convergence of proteins. *In.* Bryson, V. & Vogel, H.J. (Eds.), *Evolving Genes and Proteins*. Academic Press, New York, USA, pp. 97–166.

APPENDIX. Specimens examined.

Western lineage.—TU 1245–46, 10872, 12506, 13011, 13221, 13223, 14667, 15204, 15205, 15208 (1, 2), 15209, 17991, 18073–75, 18076 (1, 2), 18077–78, 18080–81, 18083-9, 18088, 18090–91, 18093–99, 18103, 18106, 18108–12, 18114–30, 18132–62, 18164–65, 24206–07; UF 6607, 18146, 21746, 117204, 155555–61, 156987; MNHN-AZ-AC-A4540.

Central lineage.—CRI 1565–73, 1575–78, 1580-84, 1586, 1601–05, 2133–39, 2344–47, 2350–51, 2354–55, 2357, 6880; TU 18163; UF 3998, 6801, 25047, 52676, 57968, 67782, 67784, 74780, 115414, 140993, 152479, 152939, 155267, 156975, 156978–79.

Eastern lineage = Suwannee.—UF 12694–95, 22267, 48437, 49967, 57967, 65903, 89890–91, 93476–77, 115413, 155551–53, 155562–63, 156980-81, 156983–86, 156988–91, 166146.