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Morphological Variation in the Endangered Alabama Red-Bellied Cooter (*Pseudemys alabamensis*) and Taxonomic Status of a Population in Mississippi

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ABSTRACT. – **A population of *Pseudemys*, belonging to the red-bellied turtle group, was recently discovered in the lower Pascagoula River and Biloxi Bay watersheds in Mississippi. It is presently state-listed as endangered and is ranked "S1" (extremely rare) and "G1" (extremely rare throughout its known range) globally by the Natural Heritage Program Network. The taxonomic status of this population remains unresolved, largely because of its close morphological affinity to the Federally endangered Alabama red-bellied cooter, *Pseudemys alabamensis* (Emydidae), from the Mobile Bay area of Alabama. We review the taxonomic history of *P. alabamensis* and address the taxonomic relationship between *P. alabamensis* and the new population, here called the Mississippi red-bellied cooter, based on shell morphology. Fourteen morphological traits were measured from *P. alabamensis* and Mississippi red-bellied cooters and compared with the syntopic river cooter (*P. concinna*). The goal was to determine if the Mississippi red-bellied cooter differs morphologically from *P. alabamensis* and if potential differences warrant taxonomic revision and description of the Mississippi red-bellied cooter as a distinct taxon. Principal components analyses followed by MANOVA and Bonferroni/Dunn post-hoc tests (using principal component scores) indicated that red-bellied cooters from Alabama and Mississippi form a distinct group that is distinguished from *P. concinna* based primarily on the ventral length of the cervical scute. MANOVA indicated differences between *P. alabamensis* and the Mississippi population with respect to dorsal width of the cervical scute. There were also differences in the dorsal width and ventral length of the cervical scute between Alabama and Mississippi populations of *P. concinna*. Hence, differences between *P. alabamensis* and the Mississippi red-bellied population probably reflect concordant clinal variation in morphology similar to that found in *P. concinna*. We do not recommend recognition of the Mississippi red-bellied cooter as a distinct taxon, rather our study indicates that *P. alabamensis* is not restricted to the Alabama Mobile Bay region and warrants recognition of *P. alabamensis* populations in Mississippi.**

KEY WORDS. – **Reptilia; Testudines; Emydidae; *Pseudemys alabamensis*; turtle; distribution; taxonomy; morphology; clinal variation; conservation; Mississippi; Alabama; USA**

The Alabama red-bellied cooter, *Pseudemys alabamensis*, was described by Baur (1893) and was previously included within the description of *Pseudemys mobilensis* (= *P. concinna mobilensis*) by Holbrook (1838) and *Ptychemys mobilensis* (= *P. c. mobilensis*) by Agassiz (1857). Neither specimen from the Gustave Kohn collection (now in the United States National Museum, USNM 20966–7) that Baur (1893) used for the description of *P. alabamensis* was specified as the holotype. McCoy and Vogt (1985) designated USNM 20966 as the lectotype: this is an adult male collected at the type locality, "Mobile Bay Ala." Subsequent to Viosca (1923), and before Crenshaw (1955) and Carr and Crenshaw (1957) returned *P. alabamensis* to species status, the Alabama red-bellied cooter was considered to be an invalid taxon and was designated as a "mutant of *P. floridana mobilensis*" (=*P. concinna mobilensis*) (Carr, 1938a), or a variant of "*P. floridana suwanniensis*" (=*P. c. suwanniensis*) (Carr, 1952). It was also included within what is now *P. nelsoni* (De Sola, 1935), or considered a subspecies of *P. rubriventris* (Stejneger, 1938; Wermuth and Mertens, 1961, 1977). Although *P. alabamensis* is now generally accepted as a valid species (McDowell, 1964; Weaver and Rose, 1967; Ernst and Barbour, 1972, 1989; Mount, 1975; Behler and King, 1979; Meany, 1979; Pritchard, 1979; McCoy and Vogt, 1979, 1985; Dobie, 1982, 1985, 1986, 1992a, 1993; Ward, 1984; Seidel and Palmer, 1991; Iverson, 1992; Seidel, 1994; Ernst et al., 1994), some investigators have indicated the possibility that it may be a subspecies of *P. nelsoni* (Crenshaw, 1955; Carr and Crenshaw, 1957; Jackson, 1978; McCoy and Vogt, 1979).

Pseudemys alabamensis (Fig. 1) is most closely allied with members of the *P. rubriventris* species complex (i.e., *P. alabamensis*, *P. nelsoni*, and *P. rubriventris*, see Seidel and Palmer, 1991; Seidel, 1994) based on the presence of a

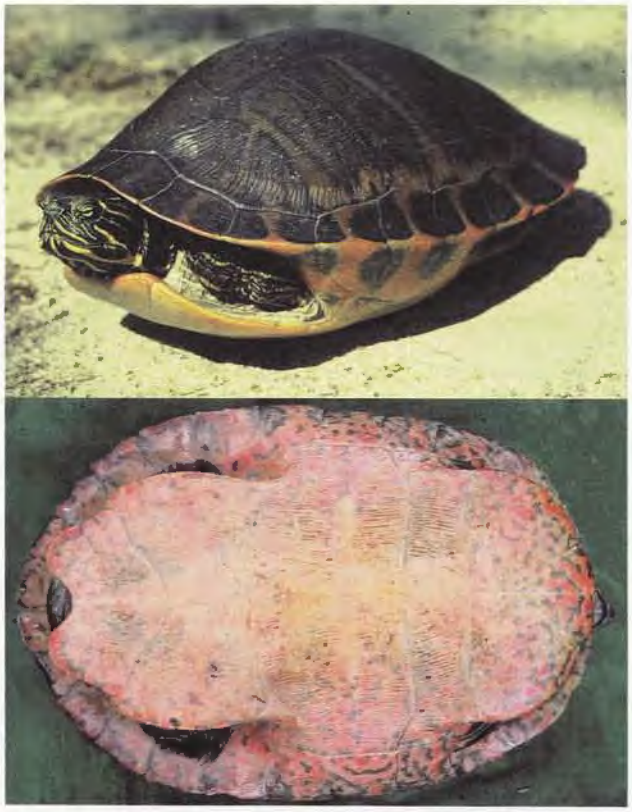

Figure 1. Female red-bellied cooter, *Pseudemys alabamensis*, from Alabama (top) and male from Mississippi (bottom). Photos by Robert H. Mount and Thomas M. Mann.

central notch in the upper jaw bordered by a cusp on each side, a vomer forming part of the triturating surface, and a prefrontal arrow formed from the meeting of the sagittal head stripe with the supratemporal stripes. The prefrontal arrow is often broken, with one or more breaks in the sagittal stripe, particularly in larger specimens (Carr and Crenshaw, 1957). Breaks in the sagittal stripes are commonly positioned such that the sagittal stripe does not join with the supratemporal stripes which typically unite at the midline dorso-posterior to the nares. Some individual *P. concinna, P. texana,* and *P. gorzugi* (Ernst, 1990) also have an upper jaw notch with cusps, a prefrontal arrow, and an orange-red plastron (e.g., Fig. 1, bottom) that suggest inclusion of these species within the *P. rubriventris* group (Ward, 1984). However, other morphological features and liver proteins link *P. alabamensis* with other members of the *P. rubriventris* complex and differentiate it from members of the *P. concinna* complex (McDowell, 1964; Weaver and Rose, 1967; Seidel and Palmer, 1991; Seidel, 1994). Seidel (1994) re-evaluated the inclusion of *P. texana, P. gorzugi,* and *P. concinna* in the *P. rubriventris* group and concluded that many features used by Ward (1984) were homoplasous features shared by *P. texana, P. gorzugi, P. concinna* and members of the *P. rubriventris* complex. Seidel (1994) recognized a monophyletic *P. rubriventris* group based on biochemical and morphological evidence.

Pseudemys alabamensis, as currently understood, is restricted to the brackish and fresh waters of the lower portion of the Mobile Bay drainage system in Mobile and Baldwin Counties, Alabama (Mount, 1975; McCoy and Vogt, 1979, 1985; Dobie, 1985, 1986, 1992a, 1993; Ernst et al., 1994; Nelson, 1995, 1996, 1997). Mount (1975) also noted the existence of a population in Little River State Park Lake, Monroe County, Alabama, that has since been extirpated (R. Mount, *pers. comm.*). Allen (1932) reported a *P. alabamensis* from the Tchoutacabouffa River (a tributary of the Biloxi Bay watershed in Harrison County, Mississippi, see Fig. 2) and numerous reports have indicated that individuals have been found as far east as Apalachee Bay, Florida (the latter specimens were assumed to be waifs, see Dobie, 1993 and Ernst et al., 1994). The identification and/or reported localities for many Florida specimens have been invalidated or questioned (Mount, 1975; Dobie, 1993; Ernst et al., 1994), hence, the current accepted range (see Mount, 1975; Ernst et al., 1994) makes *P. alabamensis* one of the most geographically restricted emydid turtles in the United States. Accordingly, *P. alabamensis* was designated as a Threatened species by the State of Alabama in 1986, and was subsequently listed as an Endangered species by the U.S. Fish and Wildlife Service (USFWS) in 1987.

In 1987, a reproductively active population of red-bellied cooters was discovered in the lower Pascagoula River, Jackson County, Mississippi (Fig. 2), that possessed morphological characteristics most similar to *P. alabamensis* (relative to other members of the *P. rubriventris* group). These characteristics include paramedian head stripes which extend anterior to the orbits, except in melanistic adults (a feature which distinguishes *P. alabamensis* from *P. nelsoni* [Carr and Crenshaw, 1957]), and a highly domed carapace (Fig. 1, top) unlike that of *P. rubriventris* (Ernst et al., 1994). Populations of red-bellied cooters in Mississippi have been regarded since 1987 as somewhat distinctive from Alabama populations of *P. alabamensis*, possibly even distinctive at the species level (Dobie, 1992c). This undescribed population was listed as Endangered by the State of Mississippi in 1992, ranked S1 (extremely rare) by the Mississippi Natural Heritage Program, and is listed by the Heritage Network as G1 (extremely rare throughout its known range) (see Buhlmann and Gibbons, 1997). Although the population has not been recognized as taxonomically distinct, it was listed as a Federal candidate "C2" species by the U.S. Fish and Wildlife Service. Throughout this paper we refer to this newly discovered and isolated population as the Mississippi red-bellied cooter (see also Buhlmann and Gibbons, 1997) in distinction to the Alabama red-bellied cooter, *P. alabamensis*.

Little has been published on the natural history of *P. alabamensis* and the Mississippi red-bellied cooter. However, information available from a number of unpublished research reports conducted from the late 1980s to the present has added significantly to our knowledge of habitat requirements, diet, behavior, reproduction, seasonal movements, and population trends of this species (see Dobie, 1991, 1992a,b; Floyd, 1995; Floyd et al., 1998; Nelson, 1995, 1996, 1997).

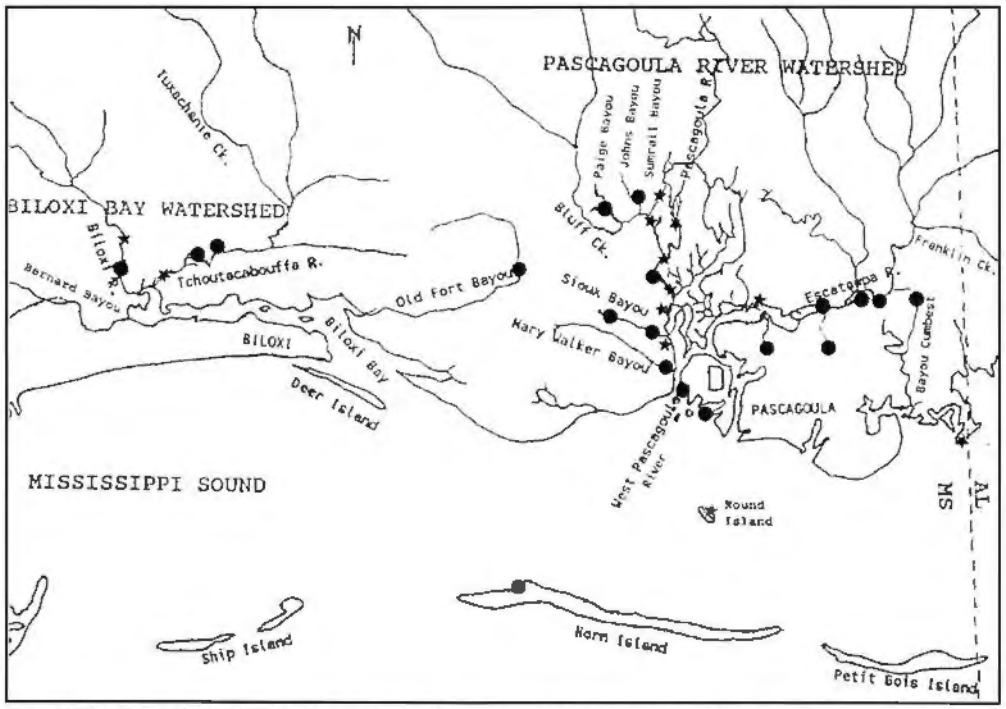

Figure 2. Collection sites for Mississippi red-bellied cooters (*Pseudemys alabamensis*) in Mississippi. Stars indicate sites at which one or more voucher specimens were collected; solid dots indicate sites where one or more specimens have been captured, measured, marked, photographed, and released. Map based on Mann et al. (2000).

Lydeard (1995) compared mtDNA (cytochrome B) sequences of *P. alabamensis* and the Mississippi red-bellied cooter and found that the two populations were indistinguishable. However, *P. concinna* and *P. alabamensis* (two species that Seidel, 1994, recognized as morphologically and biochemically distinct) also could not be distinguished from each other based upon mtDNA. Hence, morphological analyses may provide new insight into the degree of divergence between the Alabama and Mississippi red-bellied cooters otherwise not detected by conservative genes (see Avise et al., 1992). Herein, we compare 14 shell characteristics of the Mississippi red-bellied cooter and Alabama red-bellied cooter (*P. alabamensis*) to determine whether these populations are morphologically distinct or not. For outgroup comparison, morphological traits were also measured from the syntopic river cooter (*P. concinna*). If differences between the red-bellied turtle populations are significant, taxonomic elevation of the red-bellied turtle population in Mississippi to species or subspecies status may be warranted.

METHODS

We measured 14 morphological traits from the carapace and plastron of 237 turtles including 111 *P. alabamensis* (33 males, 78 females) from the lower Mobile drainage system in Mobile and Baldwin Counties, Alabama, and 22 Mississippi red-bellied cooters (12 males, 10 females) from several tributaries in the Pascagoula River and Biloxi Bay watersheds in Harrison and Jackson counties, Mississippi (see Fig. 2). We also measured 79 *P. concinna* (38 males, 41 females) from the Mobile drainage, Alabama, and 25 *P. concinna* (9 males, 16 females) from the Pascagoula, Biloxi Bay, and Jourdan River drainages in Mississippi. Most data were obtained from live animals collected in the summers of 1992 and 1993, but skeletonized museum specimens were also measured. A list of those specimens is provided in the Appendix. Shells of some individuals had morphological anomalies and/or deformities and were excluded from the analyses, as were individuals damaged by automobiles, motor boat propellers, and/or alligators (*Alligator mississippiensis*). Furthermore, we only used data from specimens with a mid-line carapace length ≥ 120 mm and examined males and females separately to reduce effects associated with ontogeny and sexual dimorphism (see also Seidel, 1994).

Morphological traits were measured with calipers and included the following standard measures used to distinguish species in the genus *Pseudemys* (see Seidel, 1994): 1) mid-line carapace length (CL); 2) shell depth at junction of 2nd and 3rd vertebral scutes (SD2-3); 3) shell depth at junction of 3rd and 4th vertebral scutes (SD3-4); 4) carapace width at junction of 5th and 6th marginal scutes (CW5-6); 5) carapace width at junction of 7th and 8th marginal scutes (CW7-8); 6) mid-line plastron length (PL); 7) dorsal width of the cervical scute (CSDW); 8) distance between epiplastron and nuchal bone (Ep-CSD); 9) mid-line epiplastral depth (EpD); 10) anal scute length (ASL); 11) distance between axillary and inguinal scutes (AX-IN); 12) ventral length of the cervical scute (CSVL); 13) dorsal length of the cervical scute (CSDL); and 14) ventral width of the cervical scute (CSVW). For illustrations of morphological characteristics see Seidel and Palmer (1991).

Statistical analyses largely followed the methods of Seidel (1994). Principal component analysis (PCA) was applied (using JMP, SAS InstituteVersion 3 and NTSYS, Applied Biostatistics Inc.), and principal component scores for all 237 turtles were calculated collectively. PCA prevented biases in the results that may occur from *a priori* assignment of groups (i.e., species and/or populations) used in discriminant analysis. Bivariate plots of principal component scores were then visually examined to subjectively assess the extent of morphological divergence or similarity among and between red-bellied cooters and *P. concinna*, and between sexes (*Pseudemys* are sexually dimorphic, see Gibbons and Lovich, 1990; Seidel, 1994). Statistically significant differences in morphology were then tested with MANOVA followed by Bonferroni/Dunn post-hoc tests to control for alpha (using Statview, SAS Institute, Version 5.0). This procedure controls for multiple comparisons by dividing alpha ($\alpha = 0.05$) by the total number of planned contrasts in the model (Toothaker, 1993).

RESULTS

The first factor (PC I) extracted from PCA was size-related (see also Seidel, 1994) and accounted for more than 74% of the variance in all male and female turtles. Eigenvectors for all morphological characters were high and positive, with CSVW, CSDW, and CSVL having the lowest loadings (Table 1). PC II accounted for 8% of the remaining variance, and PC III accounted for approximately 7%. Thus, principal components I, II, and III collectively accounted for 89% of the total variance among male and female turtles (Table 1). No individual principal component score contributing to the remaining variance was greater than 2.5%. Visual examination of PC I versus PC II bivariate plots for all male and female individuals indicated that PC II readily allowed for differentiation between red-bellied cooters and *P. concinna* (Fig. 3; MANOVA $F_{3,233} = 289$, $p < 0.0001$). PC II was primarily related to CSVL (Table 1).

Table 1. Results obtained from principal components analyses (for PC I, PC II, and PC III only). Characters are described in Seidel and Palmer (1991).

	PC I	PC II	PC III
Eigenvalue:	10.4239	1.1567	0.9343
Percent:	74.4568	8.2628	6.6741
Cumulative Percent:	74.4568	82.7196	89.3936
Eigenvectors:			
Carapace Length	0.9696	0.1466	0.0824
Shell Depth 2-3	0.9762	-0.0630	0.0453
Shell Depth 3-4	0.9729	-0.0537	0.0729
Carapace Width 5-6	0.9663	0.1635	0.0815
Carapace Width 7-8	0.9559	0.2214	0.0791
Plastron Length	0.9857	0.0744	0.0669
Cervical Scute Dorsal Width	0.5210	-0.0970	-0.7815
Epiplastron-Nuchal Distance	0.8931	0.2274	0.1081
Epiplastral Depth	0.8280	-0.3848	0.0228
Anal Scute Length	0.9079	0.0605	0.0344
Axillary-Inguinal Distance	0.8870	0.0458	0.1615
Cervical Ventral Length	0.5050	-0.8105	-0.0491
Cervical Dorsal Length	0.8410	-0.2525	0.1649
Cervical Ventral Width	0.6613	0.3340	-0.4960

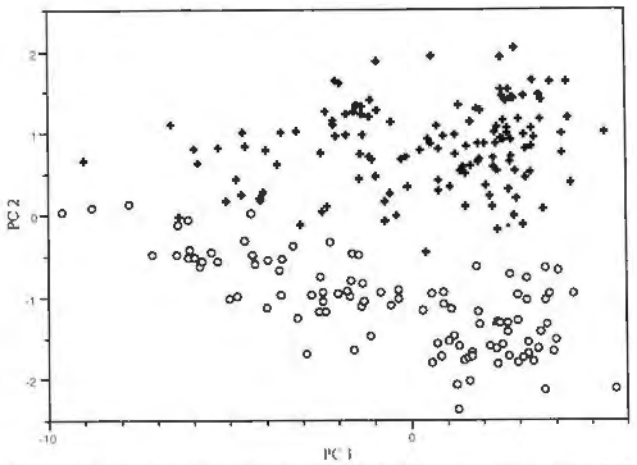

Figure 3. Bivariate scatterplot of principal component scores (PC I and PC II) for red-bellied cooters (*P. alabamensis*) from Alabama and Mississippi compared with river cooters (*P. concinna*) from Alabama and Mississippi. Crosses = *P. alabamensis*, open circles = *P. concinna*.

Examining each species separately, bivariate plots (Fig. 4) indicated that PC I separated male and female red-bellies (PC I is a combination of characters related to size, see Table 1). This was not surprising because females are larger than males (MANOVA $F_{14,118} = 25$, $p < 0.0001$, for red-bellied cooters from Alabama and Mississippi analyzed collectively). Similarly, bivariate plots of PC I, II, and III for *P. concinna* from Alabama and Mississippi (Fig. 5) indicated that PC I separated males from females (MANOVA $F_{14,189} = 27$, $p = < 0.0001$). Hence, all subsequent analyses were run separately for each sex (see also Seidel, 1994).

Visual examination of bivariate plots of PC I, II, and III indicated that there were no discrete differences between male or female *P. alabamensis* when compared, respectively, to male and female Mississippi red-bellied cooters (Fig. 4). However, Bonferroni/Dunn tests (adjusted $\alpha = 0.025$) following MANOVA (using all male turtles combined [$n = 95$] and all female turtles combined [$n = 142$]) indicated a significant difference for PC III between female *P. alabamensis* versus female Mississippi red-bellied cooters ($p = 0.007$), and a marginal difference between male *P. alabamensis* versus male Mississippi red-bellied cooters ($p = 0.0259$). PC III was primarily related to CSDW (see Table 1). No detectable differences were found for PC I or PC II for males or females ($p \geq 0.09$).

Visual examination of bivariate plots of PC I, II, and III indicated no obvious differences between male or female *P. concinna* in Alabama when compared, respectively, to male or female *P. concinna* in Mississippi (Fig. 5). However, Bonferroni/Dunn tests (adjusted $\alpha = 0.025$) following MANOVA (using all male turtles combined [$n = 95$] and all female turtles combined [$n = 142$]) indicated a significant difference with respect to PC II and PC III for female *P. concinna* from the two localities (PC II, $p = 0.004$; PC III, $p = 0.01$), but no significant difference in either parameter for male *P. concinna* in Alabama and Mississippi ($p \geq 0.2$). PC

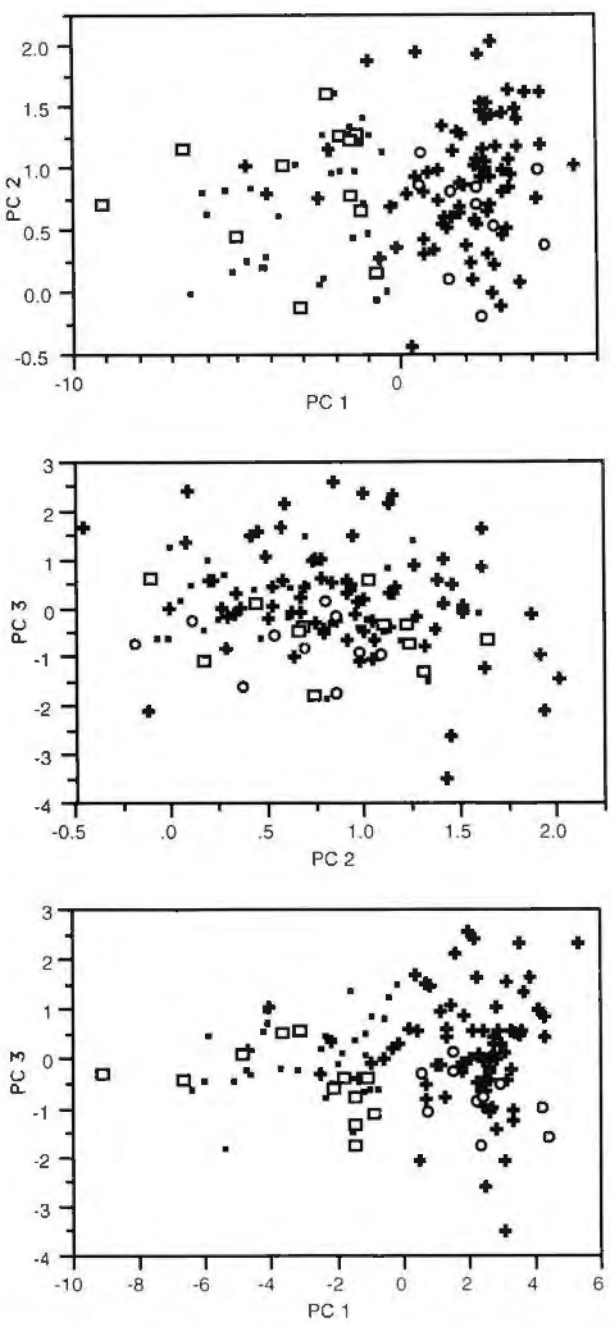

Figure 4. Bivariate scatterplots of principal component scores (PC I, PC II, and PC III) for red-bellied cooters (*P. alabamensis*) from Mississippi and Alabama. Crosses = females from Alabama, solid squares = males from Alabama, open circles = females from Mississippi, open squares = males from Mississippi.

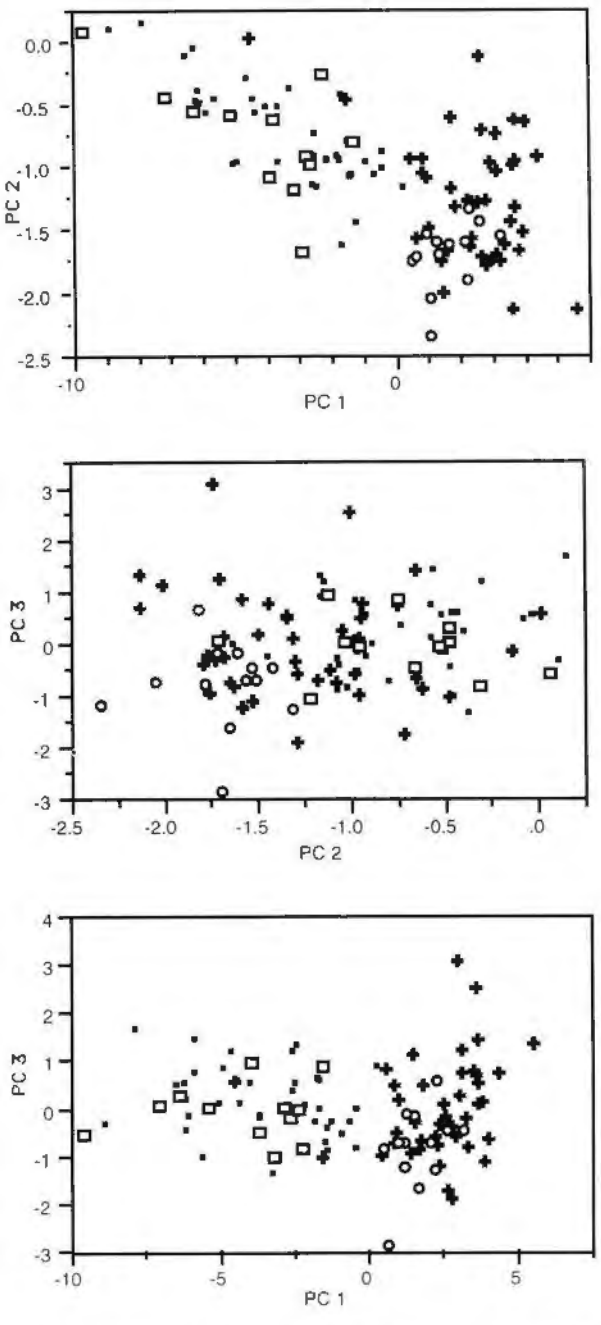

Figure 5. Bivariate scatterplots of principal component scores (PC I, PC II, and PC III) for river cooters (*P. concinna*) from Alabama and Mississippi. Crosses = females from Alabama, solid squares = males from Alabama, open circles = females from Mississippi, open squares = males from Mississippi.

II was primarily related to CSVL, and PC III primarily related to CSDW (see Table 1). There were no detectable differences for PC I (components related to overall body size, see Table 1) for male or female *P. concinna* from the two localities ($p \geq 0.3$).

DISCUSSION

Differences in the cervical scute detected between *P. alabamensis* and the Mississippi red-bellied cooter do not warrant recognition of the Mississippi population as a distinct taxon given that *P. concinna* from the same two regions exhibits similar morphological differences. Rather, *P. alabamensis* and *P. concinna* appear to be somewhat contiguous throughout Alabama and Mississippi and exhibit similar clinal variation in morphology. Broad coastal dispersal seems likely based on numerous reports of oceanic *P. alabamensis* (i.e., waifs) occurring along the coast of Alabama and Mississippi (and in some cases beyond the regions we sampled, see Dobie, 1993, and Ernst et al., 1994), distances moved by radio-telemetered *P. alabamensis* (Nelson, 1996), and evidence that *P. alabamensis* is highly

tolerant to seawater conditions (i.e., the existence of barnacles, *Balanus improvisus*, on a number of specimens we collected, and records of an individual collected on Horn Island, a distance > 10 km from the mainland, see Fig. 2).

Our current understanding of the distribution of *P. alabamensis* in Mississippi is provided in Fig. 2. Based upon records for the western–most Alabama population in Bayou La Batre, Alabama, and the eastern-most Mississippi specimen found on South Rigolets Island, Mississippi, it appears that Alabama and Mississippi populations may only be separated by a linear distance of approximately 16 km. However, the Rigolets Island record consisted of a single dead hatchling that was probably a waif from the Bayou Cumbest area. The shortest aquatic route from Bayou La Batre to the well documented populations at the mouth of the West Pascagoula River is approximately 37 km.

Fossil evidence is inadequate to assess whether *P. alabamensis* formerly existed in river systems outside its current range. Similarly, surveys may not have noted the contemporary existence of this turtle in other river systems due to its rarity and the difficulty in distinguishing it from *P. concinna*, or alternatively, it may have been recognized, but classified as a variant of *P. concinna* (see above). Therefore, it is impossible to determine whether the present range of *P. alabamensis* is expanding or has been decreased through extirpation from a formerly more extensive range.

We recommend that the Mississippi red-bellied cooter population continue to be Federally protected under the U.S. Endangered Species Act as a disjunct population of the Endangered Alabama red-bellied cooter, *P. alabamensis*.

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APPENDIX

Museum specimens examined: *Pseudemys alabamensis* (Alabama): AUMP 1906, 3840, 3971-4, AUM 6281, 9957, 10072, 10157, 11598-9, 11601, 11603, 11608, 11813, 12544, 12580, 12591, 16870-1, 17032, 19362, USA 1501-2; *Pseudemys alabamensis* (Mississippi): AUMP 1759, 3084, AUM 33582, 33629, 33698, 33743-6; *Pseudemys concinna* (Alabama): AUM 2523, 6300-1, 9588-9, 9958, 10146-7, 10305, 11600, 11604-7, 11610, 11815, 16351, 19347; *Pseudemys concinna* (Mississippi): AUM 14279-80, 33699, 33702-7, 33709, 33739, AUMP 1755-7.

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