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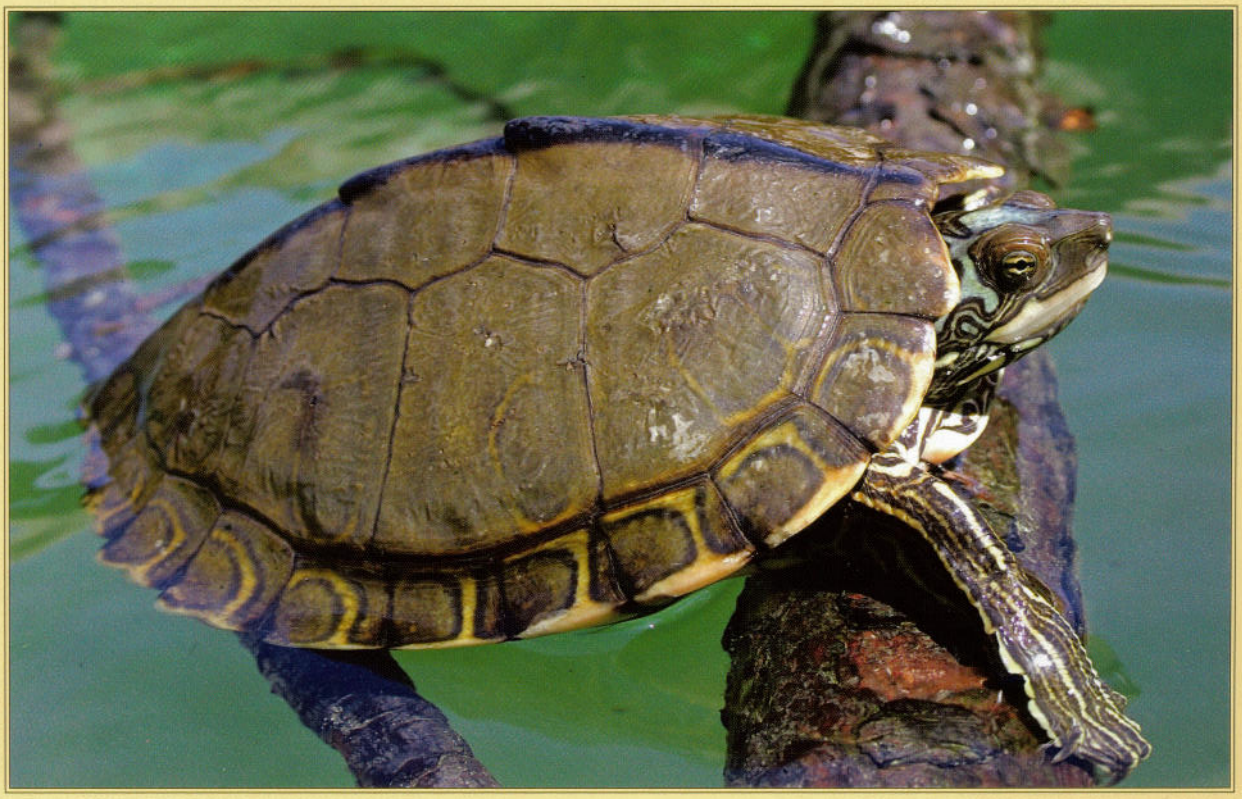

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Genetic and Morphological Variation Between Populations of the Pascagoula Map Turtle (*Graptemys gibbonsi*) in the Pearl and Pascagoula Rivers with Description of a New Species

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ABSTRACT. – **Cryptic or undescribed species pose a major problem in conservation biology. Managing multiple unresolved taxa collectively as a single entity could precipitate the loss of unrecognized genetic variation and unique populations and, possibly, lead to extinction of undiscovered or unrecognized taxa. In contrast to other species in its clade, the Pascagoula map turtle (*Graptemys gibbonsi*), as currently recognized, is not confined to a single major river system (or a cluster formed by a major river and adjacent minor drainages) but occurs in two major river systems, the Pearl and Pascagoula Rivers. We analyzed *G. gibbonsi* samples from both rivers for the first time in a morphological and molecular assessment of the taxonomic status of this poorly studied species. We compared the extent of genetic differentiation (mitochondrial DNA; mtDNA) between *G. gibbonsi* populations with members within the *pulchra* clade and between *Graptemys oculifera* and *Graptemys flavimaculata*. We found significant carapace pattern variation and morphological differentiation between the Pearl and Pascagoula river samples of *G. gibbonsi*. Our mtDNA sequences showed greater genetic differentiation between *G. gibbonsi* samples from the Pearl and Pascagoula rivers than between two recognized and reciprocally sympatric species, *G. oculifera* (Pearl River) and *G. flavimaculata* (Pascagoula River), but revealed only a modest degree of differentiation when compared to other members of the *pulchra* clade. Based on the degree of differentiation in 1) morphology, 2) color patterns, and 3) mtDNA, in addition to their 4) allopatric distributions, we describe a new species from the Pearl River, restricting the species *G. gibbonsi* to the Pascagoula River.**

KEY WORDS. – **Reptilia; Testudines; Emydidae; turtle; *Graptemys*; systematics; taxonomy; conservation; mitochondrial DNA; morphometrics; Mississippi; Louisiana; USA**

The phenomenon of cryptic or unrecognized species has been identified as a major concern in conservation biology (Lovich and Gibbons 1997). For example, in the case of the tuatara (*Sphenodon*), the failure to recognize intraspecific genetic variation between island populations almost led to the extinction of a unique form (Daugherty et al. 1990). Compounding management concerns is that, even after the recognition of cryptic taxa, there usually is a lack of basic ecological knowledge for newly described species because earlier work on a group within broad-ranging taxa was typically assumed to be applicable to the rest of its range. These scenarios are especially relevant to the southeastern United States where researchers are still describing new species from taxa previously believed widespread (e.g., *Percina*—Williams et al. 2007; *Pseudacris*—Lemmon et al. 2008).

The Alabama map turtle (*Graptemys pulchra*, as formerly recognized) is an example of a widely distributed superspecies complex that was divided by Lovich and McCoy (1992) into three species (*Graptemys ernsti*, *Graptemys gibbonsi*, and *G. pulchra*) based on morphological data and pattern variation. Along with *Graptemys barbouri*, these four species make up the *pulchra* clade of the genus (Lamb et al. 1994). All members of the *pulchra* clade, as currently recognized, possess restricted distributions, limited to only one or a few major coastal basins along the eastern Gulf of Mexico in the southeastern United States (although some species have recently been documented to occur in adjacent minor river systems as discussed below; Fig. 1). *Graptemys pulchra* inhabits the massive Alabama River basin in Alabama, Mississippi, and Georgia (Lovich and McCoy 1992). *Graptemys barbouri* is largely confined to the Apalachicola River system, with recent records from adjacent smaller drainages just to the east and west of this major system (Ernst and Lovich 2009) in Florida and southern Georgia (Sanderson and Lovich 1998). Populations of *G. ernsti* are known from the Escambia River system in southern Alabama and the panhandle of Florida, with recent records from portions of a smaller drainage immediately to the east, the Choctawhatchee River (including the Pea River tributary; Ernst and Lovich 2009). Also, the latter drainages (and others to the east) host recent records of *G. barbouri* (Enge and Wallace 2008) along with putative hybrids between the two species (Godwin 2002). Additional work is ongoing to verify the taxonomic status of these new records (J. Godwin, *pers. comm.*). In contradiction to the drainage-specific (or drainage cluster-specific) endemism (as detailed above) that typically characterizes Gulf Coast species in the genus with

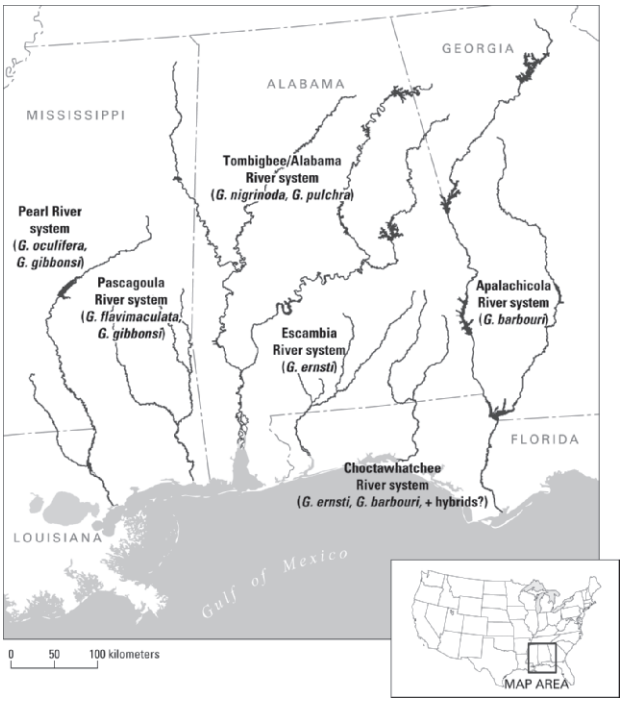

Figure 1. As currently recognized, these five coastal drainages are inhabited by seven *Graptemys* species: 1) *Graptemys oculifera* in the Pearl River; 2) *Graptemys flavimaculata* in the Pascagoula River; 3) *Graptemys gibbonsi* (sensu lato) in both the Pearl and Pascagoula rivers; 4) *Graptemys nigrinoda* in the Mobile Bay Basin; 5) *Graptemys pulchra* in the Mobile Bay Basin; 6) *Graptemys ernsti* in the Escambia Bay drainages; and 7) *Graptemys barbouri* in the Apalachicola River. The taxonomy of the *Graptemys* specimens from the Choctawhatchee River drainage is incompletely resolved.

restricted distributions, *G. gibbonsi* inhabits both the Pearl and Pascagoula river systems in Louisiana and Mississippi. As such, at the time of its description, *G. gibbonsi* was the only species in the *pulchra* clade not restricted to a single major drainage system (Lovich and McCoy 1992, 1994).

Interestingly, the distribution of *G. gibbonsi* overlaps that of two congeneric sister species, *Graptemys oculifera* and *Graptemys flavimaculata*, in the Pearl and Pascagoula rivers, respectively. Both mitochondrial DNA (mtDNA; Lamb et al. 1994; Ennen et al. in press) and morphological (Cagle 1954) data have confirmed the sister status of these two species (for additional confirmation, see Stephens and Wiens [2003], but for a slightly different view see, Wiens et al. [2010]). Sea-level fluctuations associated with glacial cycles are likely the main mechanism behind speciation within the genus *Graptemys* (Wood 1977; Lovich and McCoy 1994; Lamb et al. 1994; Walker and Avise 1998). The geologic/hydrologic history that led to the isolation and divergence of *G. oculifera* and *G. flavimaculata* presumably also would have influenced the evolution of *G. gibbonsi* as well, but the extent is not well understood. Lovich and McCoy (1992) found limited differentiation between *G. gibbonsi* populations in the Pearl and Pascagoula drainages in key morphological characters relevant to distinguishing species within the *pulchra* clade and suggested that they had been isolated for a relatively short period of time. This raises the question of how the geological history of the Pearl and Pascagoula rivers could result in speciation of the *G. oculifera/flavimaculata* ancestor yet not produce a similar degree of divergence between *G. gibbonsi* populations in these same rivers. One possibility is that the evolutionary forces (e.g., natural and sexual selection or genetic drift) that shaped speciation in *G. oculifera/flavimaculata* did not influence Pearl and Pascagoula populations of *G. gibbonsi* in the same manner. Alternatively, these populations of *G. gibbonsi*, although morphologically similar, could represent ''cryptic'' or ''covert'' species. This phenomenon is taxonomically widespread (e.g., salamanders, Larson 1984, 1989; Tilley and Mahoney 1996; fishes, Kreiser et al. 2001), especially in species with broad distribution. In addition, cryptic taxonomic units are well known in turtles (Russello et al. 2005; Spinks and Shaffer 2005; Fritz et al. 2006), including turtle species with distributions along the Gulf Coast of the United States (Roman et al. 1999).

Our goal was to use pattern variation and morphological characters along with molecular data (mtDNA) to assess the taxonomic status of *G. gibbonsi* populations in the Pearl and Pascagoula rivers. First, we reexamined morphological data and pigmentation patterns by focusing on specimens from the Pearl and Pascagoula rivers because, as a whole, differences among members of the clade have been established already (Lovich and McCoy 1992) and accepted (Turtle Taxonomy Working Group 2007, 2009; Fritz and Havăs 2007). Second, we used mtDNA sequences to compare the extent of genetic differentiation between *G. gibbonsi* samples with that found between *G. flavimaculata* and *G. oculifera*, the sister species inhabiting the same drainages. Third, mitochondrial sequence data were also used to compare the extent of genetic differentiation between populations of *G. gibbonsi* with that found in recognized species within the *pulchra* clade. Low levels of divergence in mtDNA among members of the family Emydidae, especially in the genera *Graptemys* and *Pseudemys*, have called into question its usefulness in resolving phylogenetic relationships (Lamb et al. 1994; Wiens et al. 2010). However, Wiens et al. (2010) noted that combined nuclear gene sequence data revealed greater levels of divergences in the genus *Graptemys* and that, when combined with mtDNA data, several of the clades within *Graptemys* (including the *pulchra* clade) were well supported. Because our main goal was not to produce a phylogeny of the *pulchra* clade, but rather to compare sequence divergence between *G. gibbonsi* populations with other recognized *Graptemys* species, our use of slower evolving mtDNA genes could be considered a conservative approach to this question. Our mtDNA comparisons provide an important extension to other studies (Lamb et al. 1994; Stephens and Wiens 2003;

Wiens et al. 2010) by including individuals from both rivers occupied by *G. gibbonsi*.

MATERIALS AND METHODS

Morphometrics. — We reanalyzed 223 *G. gibbonsi* museum specimens at Auburn University and the Carnegie Museum of Natural History from the Pearl and Pascagoula rivers using the same data in our analyses as used by Lovich and McCoy (1992), with the addition of several new quantitative and qualitative variables from a subsample of specimens (Appendix 1). All measurements followed Lovich and McCoy's (1992) methodology. Lovich and McCoy (1992) demonstrated that the following quantitative colorimetric variables were important for discriminating among members of the *pulchra* clade, when the sexes were analyzed separately because of sexual size dimorphism: 1) width of yellow pigment on the dorsal surface of the fifth marginal scute (MPIG); 2) width of dark pigment on the ventral surface of the fifth marginal scute (WLMP); and 3) the length of the postorbital block (LPOB). To scale for body size differences, the first two variables were divided by the width of the fifth marginal (MWID), and LPOB was divided by carapace length (CL). In addition, we measured the length of the yellow pigment bar on most posterior marginal scutes (right and left 12th marginals). This measurement (PL12) was taken from the carapace margin on both scutes to the proximal extension of the pigment bar that extended away from the margin of the carapace. To scale for body size differences, we divided measurements for PL12 by the length of each scute along an axis perpendicular to the carapace margin and the seam between each marginal scute and the most posterior vertebral scute. In some specimens, pigment length was equal to scute length for a ratio of one, but in the vast majority of specimens, it was less than one. To accommodate for asymmetry, we averaged the two ratios to generate a new variable (PL12M).

Like Lovich and McCoy (1992), we used morphometric plastron scute measurements to compare populations in both rivers. Lengths of the gular, humeral, pectoral, abdominal, femoral, and anal scutes were divided by plastron length (see also Lovich and Ernst 1989; Lovich et al. 1991; Ernst et al. 1997). All ratio data were arcsine-square-root transformed, continuous data were log transformed to meet the assumptions of normality, and all measurements follow Lovich and McCoy (1992). We used both univariate analysis of variation (ANOVA) and multivariate analysis of variation (MANOVA) to compare means among the Pearl and Pascagoula river samples of *G. gibbonsi*. Also, we conducted various discriminant function analyses to examine classification accuracy for specimens according to drainage (Pearl vs. Pascagoula).

We reanalyzed the following qualitative variables: 1) presence or absence of supraoccipital spots (SUPOC; i.e., bulbous anterior expansions of the dorsal paramedian neck stripes); 2) whether or not postorbital blotches (POB) were connected to the interorbital blotch (IOB); and 3) presence or absence of a three-pronged, light colored ''nasal trident'' above the nostrils. Again, we selected these variables because Lovich and McCoy (1992) demonstrated their discriminatory power within the *pulchra* clade overall. Two new qualitative variables were scored for the specimens we re-examined. The first was the presence or absence of a complete black vertebral stripe (VS); even if the color of the stripe graded from black to brown, but was still visibly continuous, it was scored as such. If the stripe was obviously broken by the ground coloration of the carapace, it was scored as broken. Also, we scored each specimen for the presence or absence of conspicuous secondary yellow pigment on the dorsal surface of the left fifth marginal scute of the turtle (2P5M). Secondary pigment was manifested as a series of narrow concentric rings associated with a wider bar of yellow pigment. An example of the presence of conspicuous secondary yellow pigment on this scute is shown in figure 5 of Lovich and McCoy (1992).

Because none of the qualitative pattern variables differed ontogenetically or between sexes (Lovich and McCoy 1992), we analyzed all size groups together. We used Yates Correction for Continuity (χ^2_c) to test for significant departures from expected values for qualitative categorical variables using two-by-two contingency table analyses. All tests were conducted in SYSTAT version 10 and were considered significant if test statistics were less than or equal to critical values for an alpha level of 0.05.

Genetics. — We acquired samples of *G. gibbonsi*, *G. flavimaculata*, and *G. oculifera* from April to November 2005 using basking traps or by hand during periods of low flow. *Graptemys gibbonsi* (sensu lato) were collected near the type locality in the Chickasawhay River at Leakesville Mississippi (31°08.999′N, −88°32.853′W), Leaf River north of Hattiesburg Mississippi (31°22.610′N, −89°16.641′W), and the Pearl River at Columbia Mississippi (31°17.177′N, −89°52.479′W). Approximately 1 mL of blood was drawn from the coccygeal vein using a heparinized 26.5-gauge needle and a 1-mL syringe. All individuals were released at the site of capture following sample collection. Samples were stored on ice for 4–6 h while in the field, centrifuged to separate plasma and blood cells and then stored at −20°C. For other species in the *pulchra* clade, R. Thomson (University of California, Davis) provided us with tail-tip samples (preserved in ethanol) from two individuals each of *G. barbouri*, *G. ernsti*, and *G. pulchra*. Both samples of *G. ernsti* were collected in the Conecuh River at River Falls, Alabama (31°20.936′N, −86°31.772′W). Likewise, both *G. barbouri* samples were collected from the Chipola River at a boat ramp near Marianna, Florida (30°0.588′N, −85°02.377′W). One *G. pulchra* sample was collected from the Tombigbee River at Tuscahoma Landing, Alabama (32°03.672′N, −88°06.646′W), whereas the other was collected from the Tallapoosa River in Elmore

County, Alabama (32°29.660′N, −86°14.230′W). Total genomic DNA was extracted with the DNeasy Tissue Kit (QIAGEN Inc, Valencia, CA) and gel checked on agarose to assess DNA quality. Sequence data for *Chrysemys picta* obtained from GenBank (AF069423) were used as an outgroup in all the phylogenetic analyses. Likewise, we obtained sequence data for *G. oculifera* and *G. flavimaculata* from Ennen et al. (in press; GenBank GQ253568–GQ253573).

Lamb et al. (1994) showed that the control region (CR) of mitochondrial genome provided better phylogenetic resolution within *Graptemys* than did cyt *b*. We amplified a larger and separate portion of the control region and a different portion of the genome (NADH dehydrogenase subunit 4 – ND4) using the primers reported by Spinks and Shaffer (2005). These mtDNA regions (i.e., control region and ND4) are commonly used and appear to be the most variable at the species levels among turtles (FitzSimmons and Hart 2007). Amplifications were conducted in a total volume of either 25 µl or 50 µl using 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.01% gelatin, 200 µM dNTPs, 2 mM $MgCl_2$, 0.5 units of *Taq* polymerase (Promega Co), 0.3 µM of each primer, 20–50 ng of template DNA, and water to the final volume. Cycling conditions consisted of an initial denaturing step of 1 min at 95°C followed by 30 cycles of 1 min at 95°C, 1 min at 50°C, and 1 min at 72°C. A final elongation step of 72°C for 3 min completed the reaction. PCR products were cleaned using the ExoSAP-IT system (USB Co, Cleveland, OH) and then used as the template in a cycle sequencing reaction with an ABI BigDye Terminator cycle sequencing kit (Foster City, CA) using the primers described above. All sequencing reactions were sephadex cleaned (Princeton Separations, Adelphia, NJ) prior to gel runs at the Iowa State University DNA Sequencing and Synthesis Facility. Sequence data were edited and aligned using Sequencher v. 4.1 (GeneCodes Co, Ann Arbor, MI).

PAUP* 4.0b10 (Swofford 2002) was used to calculate pairwise uncorrected *p* distances between all haplotypes within the *pulchra* clade. The degree of congruence in the phylogenetic signal of the control region and ND4 data sets was examined using the incongruence length difference test as implemented by PAUP* (Farris et al. 1994). Phylogenetic relationships among members of the *pulchra* clade were inferred using maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses. The maximum parsimony analysis was performed by PAUP* with a branch-and-bound search, and the initial upper bound was calculated by stepwise addition. The most appropriate model of sequence evolution for the ML analysis was selected by ModelTest v. 3.5 (Posada and Crandall 1998) as a HKY + G model with a Gamma distribution shape parameter of 0.0137. A Bayesian inference of the phylogeny was performed using MrBayes v. 3.1 (Ronquist and Huelsenbeck 2003). Tree space was explored starting with a random tree and employing two independent runs of four Markov chains of 1,000,000 generations, each sampled every 100 generations. Plots of log-likelihood scores versus generation time were examined to ensure that each run had reached stationarity, and the first 2500 trees were then discarded as burn-in. Phylogenetic support was assessed through bootstrapping (Felsenstein 1985) with 1000 rounds of resampling for the MP and ML analyses. The majority-rule consensus of the 7500 trees saved by the Bayesian analysis was used to obtain the posterior probabilities of each clade.

RESULTS

Morphometrics. — The MANOVA of the colorimetric quantitative variables originally used by Lovich and McCoy (1992) confirmed significant differences between Pearl and Pascagoula river samples of female *G. gibbonsi* for arcsine-square-root transformed MPIG/MWID, WLMP/MWID, and LPOB/CL variables simultaneously (Wilks' lambda = 0.569; $F = 11.347$; df = 3, 45; $P < 0.001$). The ANOVA results were significant for the first two variables (*P*-values) but not for the third ($P = 0.289$). Males also exhibited significant MANOVA results (Wilks' lambda = 0.527; $F = 16.436$; df = 3, 55; $P < 0.001$). Again, ANOVA results for males were significant for the first two variables (*P*-values) but not for the third ($P = 0.369$). Discriminant analyses using these three transformed colorimetric quantitative variables above correctly classified 86% of males and 80% of females.

Re-examination of specimens demonstrated the utility of using the mean ratio of pigment length on the 12th marginal scutes divided by scute length (PL12M) to discriminate between specimens from the Pearl and Pascagoula rivers. Like our qualitative pattern variables, PL12M did not differ among males, females, and juveniles; thus, data were combined for analysis. For all specimens from the Pascagoula River, the mean ratio of the right and left 12th marginal scutes' (PL12M) pigment length to scute length was 86.2% with a median of 92.2% (Table 1). Specimens from the Pearl River possessed much shorter mean pigment bar ratios (51.4%) for both right and left 12th marginal scutes and a median of 40.6% for both scutes combined (Table 1). Two-sample *t*-tests (estimating separate variances) demonstrated significant differences for transformed PL12 measurements between Pearl and Pascagoula river specimens whether based on the 12th right marginal scute ($t = 7.821$, df = 106.9, $P < 0.001$), the 12th left marginal scute ($t = 8.619$, df = 113.4, $P < 0.001$), or the average (PL12M) between the two scutes ($t = 8.74$, df = 108.7, $P < 0.001$).

Because the ANOVA for LPOB/CL was not significant for females using the colorimetric variables (MPIG/MWID, WLMP/MWID, and LPOB/CL) previously used by Lovich and McCoy (1992), we replaced the latter with PL12M in a new discriminant function including arcsine-square-root transformed MPIG/MWID,

Table 1. Summary of major size-scaled and qualitative pattern differences between *Graptemys gibbonsi* (sensu lato) populations, based on the subsample of specimens listed in Appendix 1. Means are followed by ranges with sample sizes in parentheses. Data for the last four variables are from Lovich and McCoy (1992: Table 1) using their larger sample. See text for abbreviations.

	River	
Variable/sex	Pascagoula	Pearl
MPIG/MWID		
Males	0.21, 0.14–0.28 (31)	0.15, 0.11–0.20 (21)
Females	0.21, 0.15–0.27 (33)	0.16, 0.10–0.25 (20)
WLMP/MWID		
Males	0.35, 0.25–0.51 (31)	0.44, 0.34–0.69 (21)
Females	0.37, 0.29–0.48 (33)	0.42, 0.37–0.51 (20)
PL12M		
Males	0.78, 0.37–1.00 (31)	0.53, 0.20–1.00 (21)
Females	0.93, 0.54–1.00 (33)	0.45, 0.15–0.90 (20)
Postorbital blotch/interorbital blotch connection	98% (88)	95% (94)
Presence of supraoccipital spot	8% (90)	2% (93)
Presence of nasal trident	66% (82)	79 (81)
Presence of subocular spot	2% (93)	0% (92)

WLMP/MWID, and PL12M. The new function correctly classified 94% of all females (Fig. 2; Table 2). For males, the highest classification accuracy (90%) was obtained by including LPOB/CL in a four-variable discriminant function with similarly transformed values of MPIG/MWID, WLMP/MWID, and PL12M (Fig. 3; Table 3).

Morphometric analysis of transformed plastron scute measurements also revealed significant differences between Pearl and Pascagoula river populations for both males (Wilks' lambda = 0.5496; df = 1, 89; approximately $F = 11.4708$; $P < 0.0001$) and females (Wilks' lambda = 0.4883; df = 6, 83; approximately $F = 13.6213$; $P < 0.0001$). Discriminant functions for plastron scute measurements classified 82% of all males correctly with misclassifications accounting for 27% of the Pascagoula River specimens and only 8% of the Pearl River specimens. Discriminant functions for plastron scute measurements classified 86% of all females correctly with misclassifications of 25% of the Pascagoula River specimens and only 4% of the Pearl River specimens.

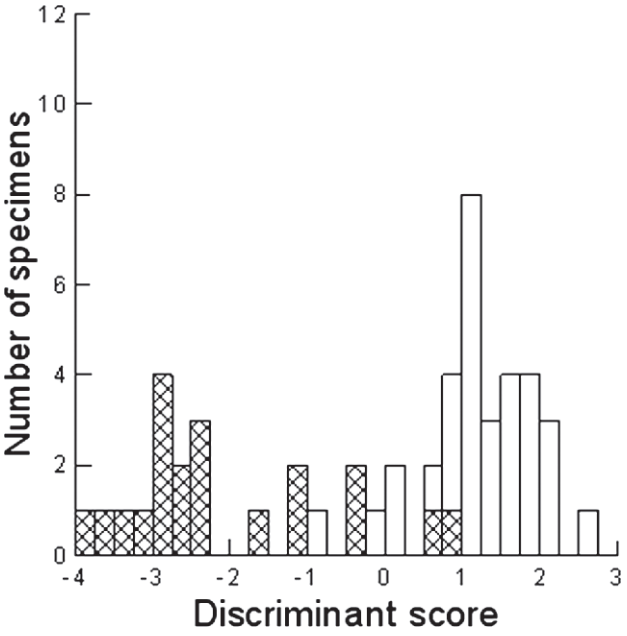

Figure 2. A histogram of female discriminant function scores (based on raw data to facilitate discrimination based on Appendix 2) showing a bimodal distribution between Pascagoula River (unfilled bars) and Pearl River (filled bars) *Graptemys gibbonsi* (sensu lato).

When we conducted discriminant function analyses using plastron scute measurements for Pearl and Pascagoula river specimens, and included other geographically proximate members of the clade (*G. ernsti* and *G. pulchra*), most of the misclassifications occurred in *G. ernsti* and *G. pulchra* for females. For males, there were significant multivariate differences among the four species (Wilks' lambda = 0.3576; df = 18, 413; approximately $F = 10.065$; $P < 0.0001$) but an overall classification accuracy of 62%. *Graptemys ernsti* was classified accurately 84% of the time, whereas the other three groups had misclassifications ranging from 36–48%. Females also showed significant multivariate differences (Wilks' lambda = 0.4644; df = 18, 515; approximately $F = 8.9128$; $P < 0.0001$) with an overall classification accuracy of 53%. However, 93% of the Pearl River turtles

Table 2. Discriminant function classification accuracy for females using the transformed variables MPIG/MWID, WLMP/MWID, and PL12M.

	Predicted river		
Actual river	Pascagoula	Pearl	Classification accuracy
Pascagoula	32	1	97%
Pearl	2	18	90%
Total	34	19	94%

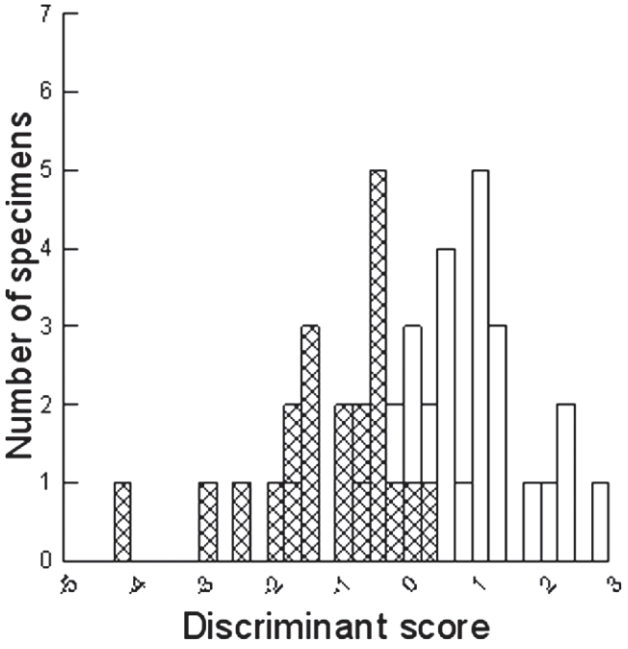

Figure 3. A histogram of male discriminant function scores (based on raw data to facilitate discrimination based on Appendix 2) showing a bimodal distribution between Pascagoula River (unfilled bars) and Pearl River (filled bars) *Graptemys gibbonsi* (sensu lato).

and 63% from the Pascagoula River were correctly classified.

Analysis of contingency tables using Yate's Correction for Continuity showed that some of the qualitative variables originally used by Lovich and McCoy (1992) for the *G. pulchra* clade were not useful for discriminating between Pearl and Pascagoula river specimens. The results for the presence or absence of SUPOC were not significant ($\chi_c^2 = 2.011$, df = 1, $P = 0.156$) between drainages. Similarly, both the comparison of POB–IOB connection and the presence or absence of subocular spots showed no significant difference between drainages ($\chi_c^2 = 0.466$, df = 1, $P = 0.495$ and ($\chi_c^2 = 0.495$, df = 1, $P = 0.482$, respectively). The results for presence or absence of a nasal trident approached significance ($\chi_c^2 = 2.903$, df = 1, $P = 0.088$), with specimens from the Pearl River marginally more likely to possess a nasal trident.

Two new qualitative pattern variables exhibited strong discriminating power. Presence or absence of an unbroken vertebral stripe (VS) differed significantly between specimens from the Pascagoula and Pearl rivers (Table 4) with those from the latter tending to have a continuous stripe more often ($\chi_c^2 = 54.679$, df = 1, $P < 0.001$). Similarly, presence or absence of conspicuous secondary marginal pigment (2P5M) differed significantly between specimens from the two rivers (Table 5). All specimens from the Pascagoula River showed some degree of secondary pigmentation, whereas most from the Pearl River did not ($\chi_c^2 = 71.517$, df = 1, $P < 0.001$).

Genetics. — For the four species within the *pulchra* clade, we obtained sequences for 24 individuals for the Control Region (CR; 658–660 bp) with the exception of one *G. pulchra* sample (521 bp) and sequences of 26 individuals for ND4 (894 bp). The number of sequences and their GenBank accession numbers for each species are provided in Table 6. The CR was more variable than ND4 (Table 7). For the CR sequence data, the uncorrected *p*-distance between the *G. gibbonsi* samples from the Pearl and Pascagoula rivers (0.013) was greater than that between the two recognized species, *G. oculifera* and *G. flavimaculata* (0.005). However, the uncorrected *p*-distance between *G. gibbonsi* from the Pearl and Pascagoula rivers was less than that found among recognized *pulchra* clade species, which ranged from 0.026–0.029 (Table 7). The ND4 sequence data showed an uncorrected *p*-distance of 0.001 between *G. oculifera* and *G. flavimaculata*, but there was no sequence divergence between the two *G. gibbonsi* populations. The ND4 uncorrected *p*-distances among other species within the *pulchra* clade were somewhat larger, but still quite low (0.006–0.007; Table 7).

In the sequence data, 95 sites were variable in the CR and 80 in ND4, of which 50 and 11 were parsimony informative, respectively. The incongruence length test

Table 4. Numbers of specimens (males, females, and juveniles) with a continuous or broken vertebral stripe.

	Vertebral stripe		
Provenance of specimen	Continuous	Broken	Total
Pascagoula River	12	60	72
Pearl River	47	9	56
Total	59	69	128

Table 3. Discriminant function classification accuracy for males using the transformed variables MPIG/MWID, WLMP/MWID, LPOB/CL, and PL12M.

	Predicted river		
Actual river	Pascagoula	Pearl	Classification accuracy
Pascagoula	24	3	89%
Pearl	2	19	90%
Total	26	22	90%

Table 5. Numbers of specimens (males, females, and juveniles) with or without conspicuous secondary marginal pigment on the upper fifth left marginal scute.

	Secondary marginal pigment		
Provenance of specimen	Present	Absent	Total
Pascagoula River	0	72	72
Pearl River	40	16	56
Total	40	88	128

Table 6. Number of individuals sequenced from each species for each gene and the number of unique haplotypes detected with their corresponding GenBank accession numbers. Sequence for *Graptemys oculifera* and *Graptemys flavimaculata* were obtained from GenBank. Species are grouped in the table based on their inclusion in either the ''*pulchra*'' or ''*pseudogeographica*'' clades.

	Control region			ND4		
	# Sequenced	# Unique haplotypes	Genbank accession #	# Sequenced	# Unique haplotypes	Genbank accession #
''*pulchra*''						
G. gibbonsi Pascagoula	9	2	GQ856224–25	11	1	GQ856234
G. gibbonsi Pearl	9	3	GQ856226–28	9	1	GQ856234
G. pulchra	2	2	GQ856222–23	2	2	GQ856232–33
G. barbouri	2	2	GQ856218–19	2	2	GQ856229–30
G. ernsti	2	2	GQ856220–21	2	1	GQ856231
''*pseudogeographica*''						
G. oculifera		2	GQ253570–71		2	GQ253572–73
G. flavimaculata		2	GQ253568–69		1	GQ253572–73

found congruent phylogenetic signal ($P = 1.0$) in the two data sets; hence, both were combined in all phylogenetic analyses. The MP analysis identified two equally parsimonious trees (L = 191, CI = 0.885, $R = 0.815$). The ML ($-\ln L = 3064.92$) and Bayesian phylogenetic analyses recovered the same basic overall topology, and the strict consensus of the two most parsimonious trees was selected to represent the phylogeny (Fig. 4). Each of the currently recognized species within the *pulchra* clade was recovered as a moderately to strongly supported monophyletic group, but there was no resolution of the relationships among the different species (Fig. 4). Internal nodes were all very weakly supported, producing a basal polytomy of the four species in the *pulchra* clade. Within *G. gibbonsi*, individuals from the Pearl River formed a strongly supported clade, but there was only weak to moderate support for the monophyly of the two haplotypes from the Pascagoula River.

Based on our analyses, we conclude that what is currently accepted as Pearl and Pascagoula populations of *G. gibbonsi* should be recognized as two separate species. Our data show that *G. gibbonsi* populations in the Pascagoula River are morphologically and genetically distinct from broad-headed *Graptemys* in the Pearl River, and recognition of these differences is compatible with the pattern of narrow distributional ranges as typical in this genus. Therefore, we describe the Pearl River population as a new species.

Graptemys pearlensis sp. nov.

Pearl River Map Turtle

Holotype. — CM 62162 (♂), Mississippi, Copiah County, Pearl River at State Highway 28, near Georgetown. Collected by T.E. Magers, 23 September 1967. D.E. Hahn collection, field number DEH 3400 (Fig. 5).

Paratypes. — AUM 21975 (♀), Mississippi, Hinds County, Pearl River; AUM 32438 (♀), Mississippi, Lawrence County, Pearl River at Monticello; CM 67474 (juvenile), 67480 (♂), Mississippi, Copiah County, Pearl River, 2 miles east of Georgetown; CM 94904 (♂), 94909 (♂), 94916 (juvenile ♂), 94940 (♂), 95050 (♀), 95055 (♀), 95059 (♂), Mississippi, Copiah County, Pearl River, Georgetown; CM 95663 (♂), Mississippi, Copiah County, Pearl River at Georgetown, Georgetown Water Park; CM 95632 (♀), Mississippi, Lawrence County, Pearl River at Monticello, Atwood Water Park; CM 95674 (♂), Mississippi, Marion County, Pearl River at Columbia Water Park.

Diagnosis. — A high-domed *Graptemys* with large females and small males, like *G. gibbonsi*, but typically with a single, generally narrower (relative to *G. gibbonsi*), vertical, yellow bar on the upper surface of each marginal scute (Fig. 6). A continuous black to brown vertebral stripe is usually present on the carapace. The yellow pigment bar on the 12th marginal scutes is usually 50% or

Table 7. Pairwise uncorrected *p*-distance values for CR (below the diagonal) and ND4 (above the diagonal). The bolded diagonal values represent the CR and ND4 intraspecific sequence divergence, respectively.

	1	2	3	4	5	6
1. *C. picta*	—	0.078	0.074	0.075	0.076	0.076
2. *G. barbouri*	0.078	**0.011/0.003**	0.006	0.006	0.006	0.006
3. *G. ernsti*	0.083	0.029	**0.002/0.00**	0.007	0.007	0.007
4. *G. pulchra*	0.088	0.026	0.029	**0.014/0.007**	0.007	0.007
5. *G. gibbonsi* Pascagoula	0.092	0.035	0.033	0.032	**0.002/0.00**	0.000
6. *G. gibbonsi* Pearl	0.140	0.040	0.040	0.035	0.013	**0.004/0.00**

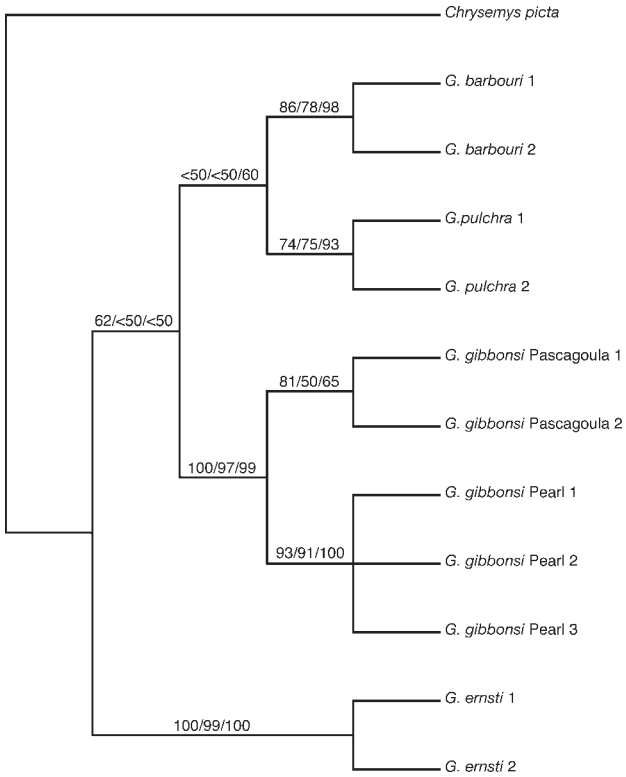

Figure 4. The strict consensus of the two most parsimonious trees (L = 191, CI = 0.885, RI = 0.815) recovered from the branch-and-bound search of the combined CR and ND4 sequence data. The support values are represented by MP and ML bootstrap and posterior probability.

less of those scute lengths along the same axis as the pigment bar (Fig. 7). When longer, the pigment bar tends to be located more distal from the seam between the 12th marginal scutes than it is in *G. gibbonsi* (Fig. 8). The head pattern is similar to that of *G. gibbonsi* and usually consists of a prominent, three-pronged, yellow nasal trident on the snout, and the postorbital blotches are connected to the interorbital blotch.

Description of the Holotype. — An adult male preserved in alcohol, with the following measurements: carapace length (maximum), 96.9 mm; carapace width, 72.2 mm; carapace height, 39.3 mm; plastron length (maximum), 86.0 mm; gular scute, 10.1 mm; humeral scute, 7.3 mm; pectoral scute, 11.2 mm; abdominal scute, 23.1 mm; femoral scute, 13.0 mm; anal scute, 17.6 mm; LPOB, 7.5 mm; MWID, 10.6 mm; MPIG 1.6, mm; and WLMP, 4.1 mm. Interorbital and postorbital blotches connected; dorsal paramedian neck stripes not contacting interorbital or postorbital blotches; nasal trident present. Carapace olive with light, indistinct circles on anterolateral corners of pleural scutes 1–3. The vertebral stripe, ranging from black to brown, is essentially complete, fading on the fifth vertebral scute, depending upon whether the specimen is wet or dry. Marginals with a narrow, vertical yellow bar on the dorsal side without conspicuous concentric rings and relatively broad black markings along ventral seams. Pigment length of the left 12th marginal scute is 3.25 mm; the length of the scute is 9.92 mm (pigment length is 32.7% of scute length); pigment length of the right 12th marginal scute is 3.8 mm; and the length of the scute is 10.04 mm (pigment length is

Figure 5. A male *Graptemys pearlensis* (the holotype, CM 62162) showing the yellow pigmentation pattern on the marginal scutes and the black vertebral stripe. Photo by J.E. Lovich. Also see cover photo of living *G. pearlensis* by C. Hagen.

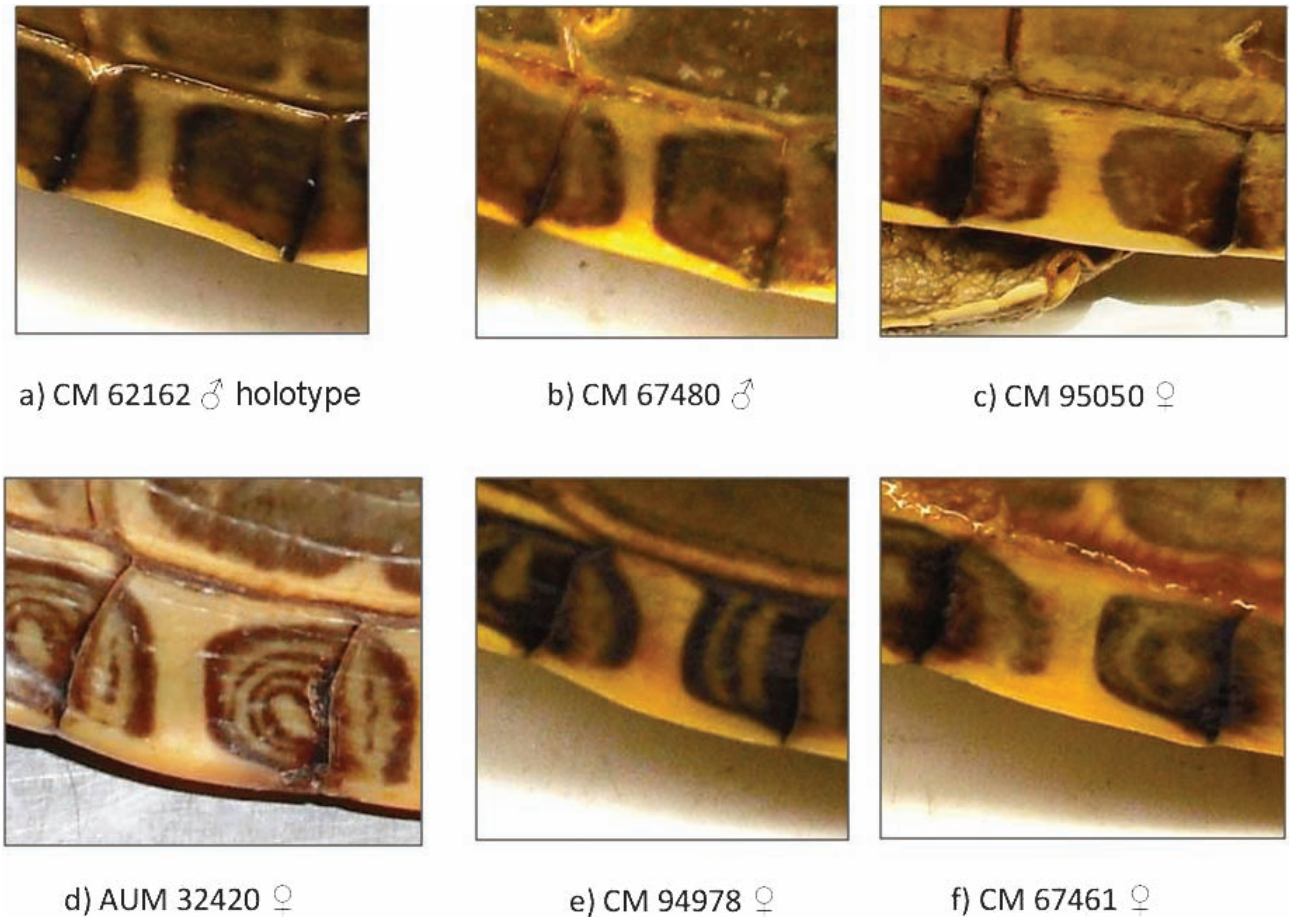

Figure 6. Variation in pigment width and presence or absence of conspicuous concentric rings for the fifth left marginal scute in *Graptemys pearlensis* (a–c) and *Graptemys gibbonsi* (d–f). Anterior is toward the left and specimen left is toward the bottom.

37.8% of scute length). Plastron yellow with black lines only along transverse seams.

Variation. — Carapace length to 295 mm in females and 120.7 mm in males. High-domed carapace with mean individual CH/CL of 0.40 (males), 0.42 (females), and 0.48 (immatures). Mean CW/CL of 0.75 (males), 0.74 (females), and 0.91 (immatures). Median keel of carapace pronounced with complete or nearly complete black stripe, most distinct anteriorly, especially on the tips of the vertebral spines. Carapace color olive with grayish cast. Relatively wide yellow rings and vermiculations on distal portions of pleural scutes. Upper marginals with relatively narrow yellow bars roughly perpendicular to carapace periphery and without conspicuous and concentric rings of secondary pigment. Mean individual MPIG/MWID of 0.18 for all specimens and 0.16 (males), 0.20 (females), and 0.20 (immatures). Mean WLMP/MWID of 0.42 for all specimens and 0.43 (males), 0.41 (females), and 0.45 (immatures). Plastron length to 250 mm in females and 106 mm in males.

A ''broad-headed'' *Graptemys*, like *G. gibbonsi*, with adult females possessing wider heads than males (for details, see Lindeman 2000; Lindeman and Sharkey 2001). Angle between sides of upper jaw viewed from above $< 90^{\circ}$, rostrum pointed. Ground color of head and limbs brown to olive with light yellow or yellowish-green stripes and blotches. Head pattern dorsally consisting of large interorbital blotch connected to large postorbital blotches; sometimes by only a thin line. Anterior portion of interorbital blotch often forming a distinct three-pronged pattern (nasal trident) in 79% of specimens examined (compared to only 65% of *G. gibbonsi*). PL12 for the right 12th marginal ranges from 14.1–100% with a mean of 51.7% and a median of 38.9%. PL12 for the left 12th marginal scute ranges from 15.0–100% with a mean of 51.0% and a median of 40.3%. PL12M, the mean of the two previous measurements ranges from 14.6–100% with a mean of 51.4% and a median of 40.6%. Mean individual LPOB/CL of 0.08 (males), 0.10 (females), and 0.08 (immatures). Dorsal neck stripes relatively broad with narrow stripes between. Underside of lower jaw with median longitudinal light stripe. Feet webbed and tail and limbs striped.

Both sexes have relatively flat plastrons. Females much larger than males (mean female CL/mean male CL = 1.93, including immatures), with conspicuously enlarged heads and hypertrophied alveolar surfaces on the jaws. Males with longer tails and the vent posterior to the margin of the carapace when extended.

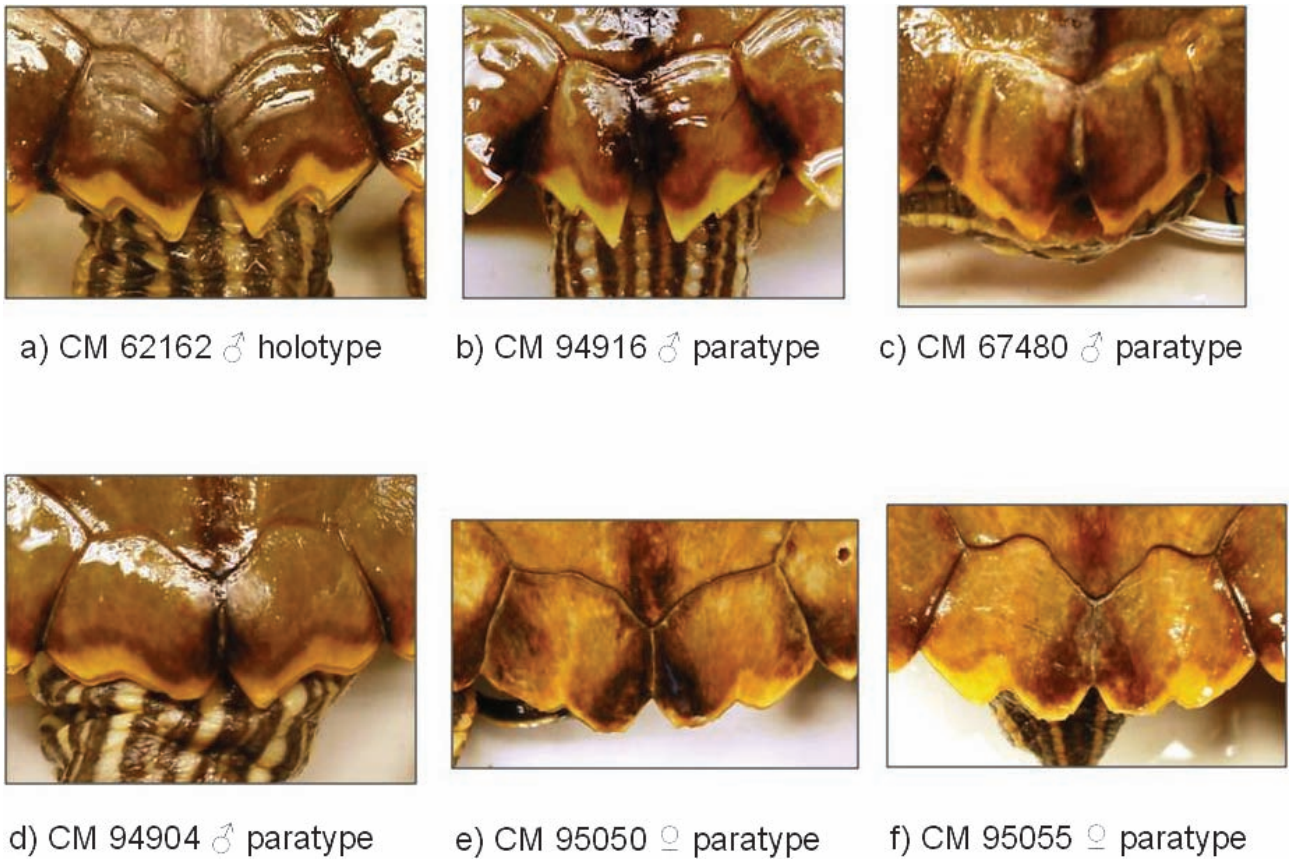

Figure 7. Variation in pigment length on the 12th marginal scutes of *Graptemys pearlensis*. Note in figure 7c that pigment trends antero-laterally when it is almost as long as the 12th marginal scute. The ratio of pigment length to scute length is usually less than 50%.

Distribution. — Found in medium-sized creeks to large rivers of the Pearl River system in Mississippi and eastern Louisiana (Lovich et al. 2009). Populations primarily occur in the Pearl and Bogue Chitto rivers, including the Ross Barnett Reservoir of the former (Boyd and Vickers 1963). Within the Pearl River system, *G. pearlensis* occur as far north as the Nanih Waiya Wildlife Management Area, northeast of Burnside, Mississippi (Neshoba County; Keiser 2000), and extend south to within 17 river miles and 23 river miles of the Gulf of Mexico in the East and West Pearl rivers, respectively (East Pearl—near Napoleon, Hancock County, Mississippi; W. Selman, *pers. obs.*; West Pearl—near Pearl River, St. Tammany Parish, Louisiana; Dickerson and Reine 1996). *Graptemys pearlensis* also occurs throughout the Bogue Chitto River in Louisiana (Shively 1999) and extends as far north as Walthall County, Mississippi (Hwy 48, west of Tylertown; MMNS 4173, 10861–10865). Also, individuals have been documented from other smaller Pearl River tributaries including the Yockanookany River (Leake Coounty, MS; Lindeman 1998), Strong River (north to Rankin County, Mississippi; P. Lindeman, *pers. comm.*), Pushepatapa Creek (Washington Parish, Louisiana; Carr and Messinger 2002), and Lobutcha Creek at Hwy 16 (Leake County, Mississippi; MMNS 15516). As noted by Lovich and McCoy (1992), Dundee and Rossman (1989) published a record of this species (as *G. pulchra*) from the Tickfaw River at US Highway 190, Livingston Parish, Louisiana. This record is still in question (Lindeman in press) because the species is unknown in the Tangipahoa, Tchefuncte, and Amite rivers, which lie between the Tickfaw River and Bogue Chitto River (Cagle 1952; Cliburn 1971). Selman and Qualls (2007) also noted a report from the Biloxi River, Mississippi (geographically between Pearl and Pascagoula Rivers), but upon investigation of suitable habitat in Harrison County, no *Graptemys* were documented in this drainage (Selman et al. 2009). However, numerous recent records of various *Graptemys* species from new localities (Ernst and Lovich 2009) suggest that our knowledge of the distribution of the genus along the Gulf Coast is incomplete. Therefore, we suspect that, in the future, individuals are very likely to be documented in other smaller, unsearched tributaries of the Pearl River system and potentially documented in other lesser-studied, independent Gulf river systems (including the Tangipahoa, Tchefuncte, Tickfaw, Amite, Jourdan, Wolf, and Biloxi rivers of Louisiana and Mississippi).

Etymology. — The specific epithet is a toponym referring to the Pearl River, the primary habitat of this species.

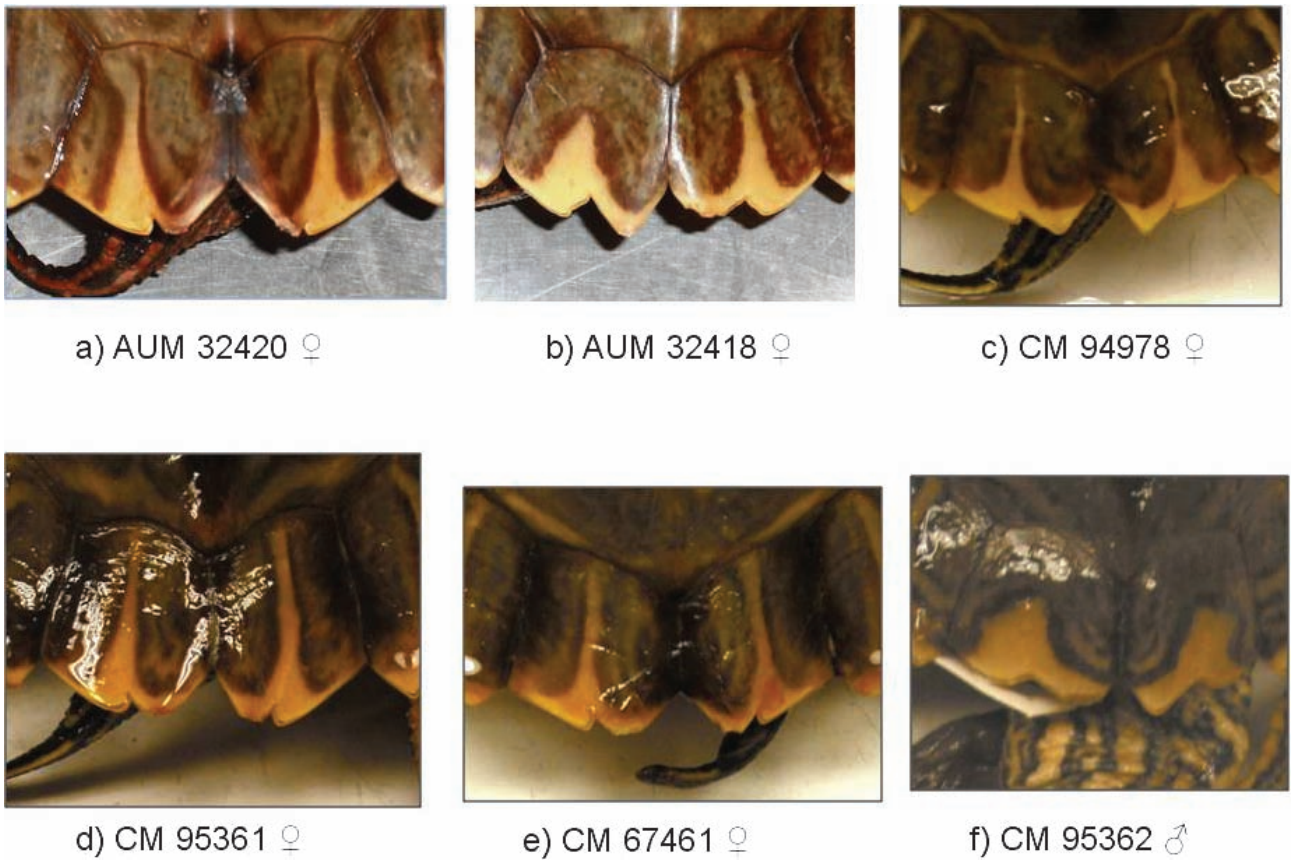

Figure 8. Variation in pigment length on the 12th marginal scutes of *Graptemys gibbonsi*. Note that the ratio of pigment length to scute length is usually much greater than 50%.

DISCUSSION

The Gulf of Mexico has experienced periodic fluctuations in sea level beginning in the Miocene (Riggs 1984; Swift et al. 1986). Endemism patterns of Gulf Coast taxa, fishes in particular, have often been interpreted as a consequence of the vicariance events associated with these sea-level fluctuations (Wiley and Mayden 1985; Swift et al. 1986). Recently this Gulf Coast allopatric speciation model has been rigorously tested in a phylogenetic framework for black basses (*Micropterus*; Near et al. 2003) and logperches (*Percina*; Near and Bernard 2004). For *Micropterus*, the bulk of the speciation events took place during the Miocene, and intraspecific diversification took place during the Pleistocene. However in *Percina*, seven of nine species diverged during the Pleistocene. Drainage-specific endemism in the genus *Graptemys*, the *pulchra* clade in particular, is apparently linked to these historical fluctuations in sea level (Wood 1977; Lamb et al. 1994; Lovich and McCoy 1992). However, Walker and Avise (1998) suggested that the genus *Graptemys* is oversplit based on comparisons of genetic divergence within other chelonian genera. They attributed this to the variety and variability of the color patterns on the heads and carapaces, which have been the focus of many species descriptions within this genus (Cagle 1953, 1954; Lovich and McCoy 1992; Vogt 1993; Ennen et al. in press). Hence, why is the genus *Graptemys* such a diverse, yet ''shallow'' lineage?

Perhaps, as Lamb et al. (1994) suggested, speciation in *Graptemys* represents a recent radiation. Explosive evolution and diversification is not unknown in aquatic vertebrates and is perhaps best exemplified by cichlid fishes in Lake Victoria. The lake holds about 200 endemic forms despite that they may be tied to a common ancestor that colonized the lake as little as 0.2–1.0 million years ago (Meyer et al. 1990). Despite extensive morphological diversification, these species show little molecular divergence. In cases of recent radiations, defining species boundaries using genealogical approaches (e.g., molecular phylogenies) remains a significant challenge (Shaffer and Thomson 2007). Alternatively, mtDNA in turtles may evolve at a slower rate relative to other vertebrates (Avise et al. 1992). Although this may not necessarily be true for all turtles (reviewed by FitzSimmons and Hart 2007), Wiens et al. (2010) did find a lower rate of divergence in *Graptemys* and *Pseudemys* relative to other emydid genera in mtDNA but not in six nuclear loci. Based on this observation, Wiens et al. (2010) suggested that speciation in *Graptemys* and *Pseudemys* may not actually be as recent as Lamb et al. (1994) had suggested.

Frost and Hillis (1990) proposed that a species could be defined as the smallest geographically constrained lineage discovered by character analysis and geographic investigation. However, they cautioned that it is undesirable to name weakly differentiated allopatric populations if there is no phylogenetic reason for doing so. Operationally, Frost and Hillis (1990) did not specify the number or kinds of differences necessary to define a species. This lack of an unambiguous criterion or cutoff for what constitutes a species causes inevitable differences of opinion, especially when considering recently radiated taxa (Shaffer and Thomson 2007). Based on the definition of the phylogenetic species concept contained in Frost and Hillis (1990), we propose that the Pearl River population of *G. gibbonsi*, in fact, represents a separate species on the basis of differences between it and the Pascagoula River population in 1) morphology, 2) color patterns, and 3) mtDNA, as well as its 4) allopatric distribution. The integrative approach, such as ours, combining morphological, molecular, and pattern data, is stronger than one using a single line of evidence (Padial et al. 2009; Türkozan et al. 2010), especially when the results are congruent. Similarly, de Queiroz (2007) suggested that the presence of any one of the properties related to operational delimitation of a species (e.g., reproductive isolation, diagnosibility, or monophyly) was evidence for the existence of a new species as long as the evidence was appropriately interpreted and demonstrated lineage separation. Recognition of both *G. pearlensis* and *G. gibbonsi* is also consistent with the phenomenon of drainage-specific (or drainage cluster-specific) endemism in the genus (Lovich and McCoy 1992).

Initial division of the *pulchra* clade by Lovich and McCoy (1992) resulted in all species but one, *G. gibbonsi*, having distributions that were restricted to single major river drainages. Lovich and McCoy (1992) noted that this exception in *G. gibbonsi* was partially congruent with the similarity of the fish fauna between the Pearl and Pascagoula rivers as described by Swift et al. (1986). However, this does not mean pattern or morphological differences were not previously noted between *G. gibbonsi* (sensu lato) in the two drainages. Tinkle (1962), Little (1973), Shealy (1976), and Lovich and McCoy (1992) all noted drainage-specific morphometric and colorimetric characteristics unique to populations in the Pearl and Pascagoula rivers. In recognition of these differences, some researchers suggested that subspecific designations might be warranted (Pritchard 1979), whereas others recognized a ''Pascagoula River form'' and a ''Pearl River form'' of *G. gibbonsi* (Vetter 2004). Conversely, Artner (2008) declared that *G. gibbonsi* was a subspecies of *G. pulchra*, along with *G. barbouri* and *G. ernsti*, but did not present any data or analyses to support his pronouncement.

Similar to these earlier studies, our morphological and pattern analyses identified the existence of physical differences between the Pearl and Pascagoula river samples. *Graptemys pearlensis* are more likely to have narrower yellow pigment bars on the upper fifth marginal scutes, wider dark pigment bars on the lower fifth marginal scute, smaller yellow pigmentation ratios on the 12th marginal scutes, an unbroken vertebral stripe, and an absence of conspicuous secondary marginal pigment compared to specimens from the Pascagoula River. Although the differences are statistically significant in multivariate space, the degree of character overlap sometimes makes it difficult to discriminate between *G. gibbonsi* and *G. pearlensis* in a specimen for which the provenance is unknown. Similar morphometric and pattern differences are seen between the turtles *Kinosternon baurii* and *Kinosternon subrubrum*, and discriminant functions are necessary to distinguish between the two, especially in some parts of their ranges (Lamb and Lovich 1990; Lovich and Lamb 1995). To make discrimination of *G. pearlensis* from *G. gibbonsi* easier, we present a simple key and function in Appendix 2.

Table 8. Uncorrected *p*-distance values from Lamb et al.'s (1994) data with CR (below the diagonal) and cyt *b* (above the diagonal) found within the *pulchra* clade, *Graptemys oculifera*, and *Graptemys flavimaculata*.

		1	2	3	4	5	6	7
1.	*C. picta*	—	0.060	0.076	0.068	0.066	0.063	0.066
2.	*G. barbouri*	0.124	—	0.015	0.013	0.010	0.010	0.010
3.	*G. ernsti*	0.107	0.044	—	0.006	0.003	0.003	0.003
4.	*G. pulchra*	0.121	0.032	0.020	—	0.003	0.003	0.003
5.	*G. gibbonsi* Pearl	0.121	0.041	0.032	0.029	—	0.000	0.000
6.	*G. flavimaculata*	0.118	0.061	0.047	0.041	0.050	—	0.000
7.	*G. oculifera*	0.127	0.047	0.041	0.029	0.047	0.017	—

The phylogenetic relationships of many *Graptemys* species remain unresolved (Lamb et al. 1994; Stephens and Wiens 2003; Wiens et al. 2010). However, the goal of including molecular data in this study was not to produce a phylogeny of the *pulchra* clade but rather to determine whether levels of sequence mtDNA divergence supported recognition of *G. pearlensis*. The lack of strong nodal support for the relationships among the ''*pulchra*'' species is congruent with previous mtDNA phylogenetic studies of *Graptemys*, where only three broad clades (*pulchra*, *pseudogeographica*, and *geographica*) have been resolved (Lamb et al. 1994; Stephens and Weins 2003), with the exception of placement of *G. caglei* (but on this point, see Wiens et al. 2010). However, Lamb et al.'s (1994) mtDNA control region sequence data supported Lovich and McCoy's (1992) recognition of *G. pulchra* (sensu lato) as three distinct species, *G. pulchra*, *G. ernsti*, and *G. gibbonsi*. Although we sequenced a different portion of the CR, our data were comparable to that of Lamb et al. (1994) in that we found similar levels of sequence divergence between these species. Lamb et al.'s (1994) uncorrected *p*-distances ranged from 0.020–0.044 (Table 8), whereas our data ranged from 0.026–0.040 for the same *pulchra* clade comparisons. The

amount of CR sequence divergence between *G. gibbonsi* and *G. pearlensis* was at the low end of the range exhibited among species in the *pulchra* clade. Thus, it is conceivable that *G. gibbonsi* and *G. pearlensis* have been separated for a relatively short period of time compared to other members within the clade, which would explain the modest molecular difference between them.

Although *G. gibbonsi* and *G. pearlensis* exhibit a lower degree of sequence divergence when compared to other species within the *pulchra* clade, these two species have a much higher degree of sequence divergence in the control region (2.6 times) compared to the other two currently recognized species of *Graptemys* in the Pearl and Pascagoula rivers, *G. oculifera* and *G. flavimaculata* (Table 6). Regardless of the reasons behind this difference in levels of sequence divergence, this comparison adds to our argument for the recognition of *G. pearlensis*. Discussion of the taxonomic status of *G. oculifera* and *G. flavimaculata* is outside the scope of this study. However, we note that when considering morphological characteristics and patterns (Ennen et al. in press) along with sequence data for six nuclear genes (Wiens et al. 2010), *G. oculifera* and *G. flavimaculata* were both distinct.

Even if the vicariant event that led to the isolation of *G. gibbonsi* and *G. pearlensis* was as recent as the Pleistocene, the allopatry of the two species, coupled with projected sea-level rise under global warming scenarios (Dasgupta et al. 2009), assures that they will remain separated for a long time to come. Despite our limited sampling within both species in the upper tributaries and lower portions of the drainages, it is unlikely genetic exchange was or will be probable over land or through estuaries even though these upper tributaries and estuaries are in close proximity. This is especially true given that smaller tributaries and estuaries are rarely occupied by *Graptemys* in Mississippi because of the unsuitable habitat (Selman and Qualls 2007, 2009). For example, *Graptemys* are highly aquatic and rarely leave the water other than for basking or, in the case of females, nesting events (Lovich and McCoy 1992; Ernst and Lovich 2009). Likewise, salt marshes are effective barriers for impeding migration of this genus (Wood 1977).

These taxonomic findings have important conservation implications as well. *Graptemys gibbonsi* (sensu lato) populations were considered Lower Risk/Near Threatened by IUCN (Lovich et al. 2009) but recently have been reported as less abundant than the two federally threatened species *G. oculifera* and *G. flavimaculata* (Lindeman 1999; Selman and Qualls 2009). Failure to recognize the Pearl and Pascagoula rivers as separate species in conservation planning could result in loss of significant evolutionarily lineages (Lovich and Gibbons 1997) in a region well known for its biological diversity (Lydeard and Mayden 1995). As such, the two species meet the primary criterion of the unified species concept of separately evolving metapopulation lineages (see de Queiroz 2007). That *G. pearlensis* can be delimited based on pattern, morphology, and mtDNA from *G. gibbonsi* are secondary, but concordant, criteria unrelated to species conceptualization per se.

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APPENDIX 1

Specimens Examined

Graptemys pearlensis (N = 56). — Mississippi: *Copiah County*, Pearl River, St Hwy 28, east of Georgetown (CM 62162–3, 67473–82, 94903–6, 94909, 94916–7, 94919–20, 94935–6, 94938–41, 94946, 95050, 95055–9, 95553, 95634, 95645–7, 95650), Pearl River, Georgetown Water Park (CM 95632), Pearl River, 25 miles south of Jackson (AUM 21975–6), Pearl River, George Town (CM 95015, 94883); *Lawrence County*, Pearl River, Monticello (AUM 25140–2, 32430, 32435, 32437–8, CM 95663–4); *Marion County*, Pearl River, Columbia Water Park (CM 95674); *Pearl River County*, Pearl River, St Hwy 26, east of Bogalusa (CM 95688); *Simpson County and Copiah County*, Pearl River, 25 miles south of Jackson (AUM 21976).

Graptemys gibbonsi (N = 72). — Mississippi: *Clarke County*, Chickasawhay River, US Hwy 45, Shubuta (CM 95879); *George County*, Pascagoula River, St Hwy 26, 2 miles east of Benndale (AUM 5966, 13657), Pascagoula River, near Lucedale (AUM 22014); *Greene County*, Chickasawhay River, Leakesville (AUM 10299, 22002–3, 22009–10, 22015, 32411, 32413–6, 32418 CM 67455–62, 94966–7, 94970–3, 94976–81, 94983, 95361–2, 95559, 95561, 95577), Chickasawhay River, 2 miles north of Leakesville (AUM 25977), Chickasawhay River, US Hwy 98 (AUM 22004–8, 22016, 31876, 32419, 32422–6, 32428–9), Leaf River, US Hwy 98, McLain (CM 95563, 95570–2); *Jackson County*, *Pascagoula* River, 9.6 km west of Wade (CM 95875); *Wayne County*, Chickasawhay River, 4.8 km west of Waynesboro (CM 67438–44, 94948–9).

APPENDIX 2

A key and discriminant function for distinguishing between *Graptemys gibbonsi* and *Graptemys pearlensis* using raw measurements. Measurements and variables are described in the Materials and Methods section and (in part) Lovich and McCoy (1992). If the discriminant score derived from the sex-specific function is less than zero, the specimen is more likely to be *G. pearlensis*. If the score is greater than zero, the specimen is more likely to be *G. gibbonsi*. Refer to Figures 3 and 4 for distribution of scores (based on raw data) relative to the two species/sexes. Discriminant scores < -1 or > 1 have a much higher probability of correctly assigning species.

Key

1. Yellow pigment bar on 12th marginal scutes $< 50\%$ of scute length and trending antero-laterally from the rear margin of the carapace, yellow bar on fifth marginal scutes narrow and without prominent concentric rings, dark vertebral stripe usually unbroken, more likely to have a nasal trident — *G. pearlensis*
2. Yellow pigment bar on 12th marginal scutes $> 50\%$ of scute length and trending antero-medially from the rear margin of the carapace, yellow bar on fifth marginal scutes wide with associated and conspicuous concentric rings, dark vertebral stripe usually broken, especially toward the posterior, less likely to have a nasal trident — *G. gibbonsi*

Female Discriminant Function

$$\text{Discriminant score} = 5.253\left(\frac{\text{MPIG}}{\text{MWID}}\right) - 8.988\left(\frac{\text{WLMP}}{\text{MWID}}\right) + 5.411(\text{PL12M}) - 1.548$$

Male Discriminant Function

$$\text{Discriminant score} = 18.336\left(\frac{\text{MPIG}}{\text{MWID}}\right) - 7.875\left(\frac{\text{WLMP}}{\text{MWID}}\right) + 0.721(\text{PL12M}) + 45.453\left(\frac{\text{LPOB}}{\text{CL}}\right) - 4.255$$

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