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Author(s): Joshua R. Ennen, Brian R. Kreiser, Carl P. Qualls, and Jeffrey E. Lovich

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## Morphological and Molecular Reassessment of *Graptemys oculifera* and *Graptemys flavimaculata* (Testudines: Emydidae)

JOSHUA R. ENNEN,<sup>1,2,3</sup> BRIAN R. KREISER,<sup>1</sup> CARL P. QUALLS,<sup>1</sup> AND JEFFREY E. LOVICH<sup>2</sup>

<sup>1</sup>Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, Mississippi 39406 USA

<sup>2</sup>U.S. Geological Survey, Southwest Biological Science Center, Flagstaff, Arizona 86001 USA

**ABSTRACT.**—The turtle genus *Graptemys* consists of 15 recognized taxa, distinguished largely on the basis of pigmentation pattern (i.e., soft tissue and shell), head size, and shell morphology. However, phylogenetic studies have shown limited sequence divergence within the genus and between *Graptemys oculifera* and *Graptemys flavimaculata* relative to most other members of the Emydidae. *Graptemys oculifera* of the Pearl River drainage and *G. flavimaculata* of the Pascagoula River drainage have been recognized as species since 1890 and 1954, respectively. However, the description of *G. flavimaculata* was based on a limited number of morphological characters. Several of these characters overlap between *G. flavimaculata* and *G. oculifera*, and no attempt was made to test for significant morphological differentiation. In this study, we reevaluated the morphological and genetic distinctiveness of *G. flavimaculata* and *G. oculifera* with (1) multivariate statistical analyses of 44 morphological characters and (2) 1,560 bp of sequence data from two mitochondrial genes (control region and ND4). The morphological and molecular analyses produced incongruent results. The principal components analysis ordinations separated the two species along a pigmentation gradient with *G. flavimaculata* having more yellow pigmentation than *G. oculifera*. Likewise, clustering analyses separated the specimens into two distinct groups with little overlap between the species. Our mitochondrial data supported previous findings of limited genetic differentiation between the two species. However, the results of our morphological analyses, in conjunction with recently published nuclear gene sequence data, support the continued recognition of the two species.

The systematics and evolutionary history of the genus *Graptemys* has long been controversial (Lovich and McCoy, 1992; Vogt, 1993; Lindeman, 2003) and remains so today. Within the largely North American family Emydidae, the genus *Graptemys* is the most speciose (Ernst and Lovich, 2009). Unlike other turtle genera that are usually morphologically conserved, *Graptemys* species have various shell or soft tissue patterns that often distinguish drainage-specific species (Walker and Avise, 1998). *Graptemys flavimaculata* (endemic to the Pascagoula River) and *Graptemys oculifera* (endemic to the Pearl River) were described by Cagle (1954) and Baur (1890), respectively. Cagle (1954) proposed several diagnostic morphological characters to differentiate the two species, including *G. flavimaculata* having (1) a broad orbital mark usually connected to a neck stripe, (2) broad yellow lines dominating the lower jaw, and (3) each costal scute with a large yellow blotch or crescent. However, several of the putatively diagnostic characters proposed by Cagle (1954) actually overlap between the species (e.g., shape of postorbital blotch, connection of neckline with postorbital blotches, and number of lines

entering the orbit). Other diagnostic characteristics consisted of additional pattern differences (e.g., width of interorbital lines, neck lines entering orbital, markings on lower jaw, and markings of extremities), but these differences were never quantified and tested statistically. Later, and without supporting data, Mertens and Wermuth (1955) included *G. flavimaculata* as a subspecies of *G. oculifera*, but this taxonomic change was neither supported by analysis nor adopted by the scientific community.

Past phylogenetic studies have not been particularly successful in resolving relationships among species in the genus *Graptemys* (Lamb et al., 1994; Stephens and Wiens, 2003), although the recent work of Wiens et al. (2010) has provided better phylogenetic resolution. Lamb et al. (1994) collected data on whole mitochondrial genome restriction sites and sequences for fragments of two mitochondrial genes (control region, 344 bp; cytochrome *b*, 380 bp). The combined data analysis only found support for three clades, which they identified as a “*pulchra*” clade, a “*pseudogeographica*” clade, and the basal *Graptemys geographica*. Although the control region data were used to identify each species, there was typically little genetic differentiation among species. Figure 5 in Lamb et al. (1994) shows only a two-base difference between *G. flavimaculata* and *G.*

<sup>3</sup>Corresponding Author. E-mail: jennen@usgs.gov

*oculifera* (uncorrected *p* distance of 0.006). The limited degree of genetic divergence among species of *Graptemys* compared to other species of freshwater turtles led Walker and Avise (1998) to propose that the genus may be oversplit. A broader study by Stephens and Wiens (2003) for the family Emydidae combined existing molecular data with a large morphological data set (>300 characters). Analysis of the combined data found that relationships among *Graptemys* species were mostly poorly resolved with weak bootstrap (62%) support for a monophyletic *G. flavimaculata* and *G. oculifera*. However, Wiens et al. (2010) found that the two species were distinctive using six nuclear loci but still possessed little divergence relative to most other members of Emydidae.

The low level of genetic divergence compared to other emydid species and lack of rigorous statistical tests of morphological differences raises questions as to the taxonomic validity of *G. flavimaculata* and *G. oculifera*. This is not just a question of academic interest because both species are federally listed as Threatened (U.S. Fish and Wildlife Service, 1986, 1991) and listed as Endangered by the state of Mississippi (Mississippi Department of Wildlife, Fisheries, and Parks, 2000). The goals of this study were to reevaluate the distinctiveness of *G. flavimaculata* and *G. oculifera* through (1) multivariate statistical analyses of a suite of morphological characters from the original species description and others used in similar studies within the genus *Graptemys* (Lovich and McCoy, 1992; Vogt, 1993) and (2) the analysis of a larger molecular data set that includes different portions of the mitochondrial genome.

#### MATERIALS AND METHODS

**Morphological.**—Preserved specimens of *G. oculifera* (55 specimens; 24 females, 31 males) and *G. flavimaculata* (93 specimens; 19 females, 74 males) were examined from the Mississippi Museum of Natural Sciences (MMNS) and the Tulane University Museum of Natural History (TU) (Appendix 1). We selected 44 characters from Cagle's (1954) description of *G. flavimaculata* and from the taxonomic literature on other *Graptemys* species (Lovich and McCoy, 1992; Vogt, 1993). The following quantitative characters were measured: carapace length (CL); carapace width (CW); width of yellow and dark pigmentation dorsally (WPIGD and WDPD) and ventrally (WPIGV and WDPV; Fig. 1) on the 5th marginal scute; width of the yellow pigmentation on the first vertebral scute (WVPIG; Fig. 1), abdominal length (AB), anal length (AN), femoral length (F), gular length

(G), humeral length (H), pectoral length (P), plastron length (PL), and plastron width (PW); width and length of the yellow blotch on the axial scute (WYAP and LYAP); width and length of the yellow blotch on the inguinal scute (WYIP and LYIP); length and width of interorbital line (LIOL and WIOL; Fig. 1); length and width of postorbital blotch (LPOB and WPOB; Fig. 1); width of the upper and lower neck lines entering the orbital (NLL and NLU; Fig. 1); width of dark line between the upper and lower neck lines entering the orbital (WBLO; Fig. 1); width of dark pigmentation between the second and fourth lines on the hind limbs (WDH); width of second (WY2F) and fourth (WY4F) yellow line on the forelimb (Fig. 1); width of second (WY2H) and fourth (WY4H) yellow line on hind limbs; and width of jaw (JW). All measurements were taken on the right side of each specimen.

The following qualitative characters (i.e., presence/absence, meristics, and categorical) were recorded: presence/absence of yellow line starting on third digit extending through elbow (3YFE); presence/absence of dorsal yellow neck line touching the postorbital blotch (DLPOB; Fig. 1); presence/absence of ventral line connecting under the chin (LLC); presence/absence of dorsal neckline extending past the interorbital line (NLIOL); presence/absence of interorbital line extending and connecting with lateral line at the nasal (NASAL); presence/absence of a U-shaped bar under the jaw (YUC); number of dorsal yellow neck lines touching the postorbital blotch (#NLPOB); number of lines entering the orbit (#NLO); number of ventral yellow lines on the hind limb where digit meets leg (#YH); number of ventral yellow lines extending from digit of hind limb through the elbow (#YHE); number of ventral yellow lines on the forelimb extending to the elbow (#YLFE); and classification of the costal scute markings (Fig. 1).

Each sex was analyzed separately to account for sexual dimorphism (Gibbons and Lovich, 1990; Lovich and McCoy, 1992). To correct for size differences within each sex, each quantitative variable was divided by carapace or plastron length, and all ratio data were arcsine-square-root transformed to meet the assumptions of normality. Principal components analyses (PCA) were performed to visualize the data for males and females in multidimensional space. To test for significant differences between *G. oculifera* and *G. flavimaculata*, we used Euclidean distances to create a dissimilarity matrix of the quantitative variables and we performed a nonparametric multiresponse permutation procedure (MRPP) with 50,000 permutations. MRPP is a resampling approach

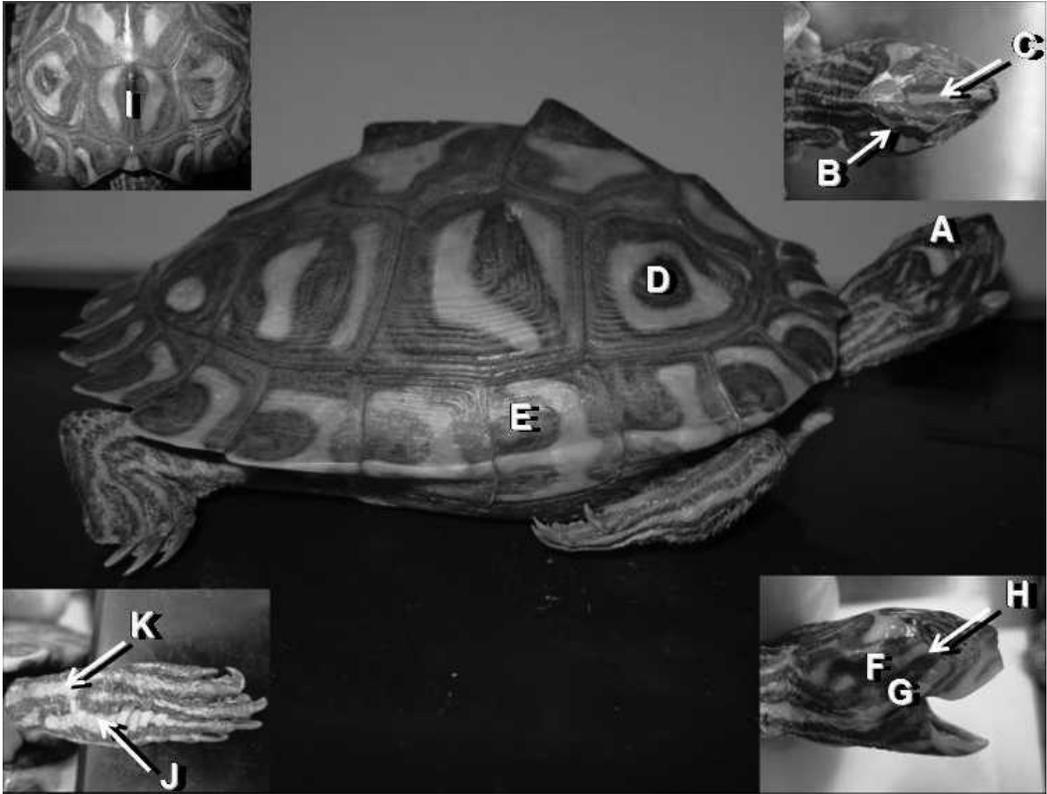


FIG. 1. Image of a female *Graptemys flavimaculata* (MMNS 005696) illustrating several characters measured for the morphological analyses: (A) length and width of postorbital blotch (LPOB and WPOB); (B) presence/absence of dorsal yellow neck line touching the postorbital blotch (DLPOB); (C) length and width of interorbital line (LIOL and WIOL); (D) classification of the costal scute markings; (E) width of yellow pigmentation dorsally (WPIGD); width of the upper (F) and lower (G) neck lines entering the orbital (NLL and NLU); (H) width of dark line between the upper and lower neck lines entering the orbital (WBLO); (I) width of the yellow pigmentation on the first vertebral scute (WVPIG); and width of second (J) and fourth (K) yellow line on the forelimb (WY2F and WY4F).

testing for a difference between groups (McCune and Grace, 2002). To determine which of the characters were driving the separation in the multidimensional space, we used the highest and lowest loading scores (i.e., absolute value of  $\leq 0.20$ ). For the qualitative variables, dissimilarity matrices were again created using Euclidean distances. These were then used in an unweighted pair group method with arithmetic means (UPGMA) cluster analyses, which when coupled with cophenetic correlation, provided a measure of how much structure was in the data. All statistical analyses were performed using R statistical software (Vers. 2.8.0, R Development Core Team, Vienna, Austria, 2008).

**Molecular.**—Blood samples from a total of 14 individuals (8 *G. oculifera*, 6 *G. flavimaculata*) were collected under the appropriate permits by W. Selman. The *G. flavimaculata* were either from the Chickasawhay River at Leakesville (31°08.999'N,

088°32.853'W;  $N = 2$ ), Leaf River north of Hattiesburg (31°22.610'N, 089°16.641'W;  $N = 2$ ) or the lower Pascagoula River (30°30.938'N, 088°36.197'W;  $N = 2$ ), whereas the *G. oculifera* were all from the Pearl River at Columbia (31°17.177'N, 089°52.479'W;  $N = 8$ ). Total genomic DNA was extracted from the blood samples with a DNeasy Tissue Kit (QIAGEN, Inc., Valencia, CA). Lamb et al. (1994) found that the control region (CR) of the mitochondrial genome had more phylogenetic signal than cytochrome *b* (cyt *b*) within *Graptemys*. We elected to examine a separate portion of the CR as well as another mitochondrial gene (NADH dehydrogenase subunit 4, ND4). Amplifications of the CR were performed with the primers of Spinks and Shaffer (2005). Likewise for ND4, we used one of the primers reported by Spinks and Shaffer (2005), but we created a new primer (ND4a; 5'-TGACT-ACCAAAGCACACGTAGAAGC-3') by modi-

fying the ND4-672 primer to match the sequence of *Chrysemys picta* (GenBank Accession AF069423) taken from GenBank.

Polymerase chain reaction (PCR) amplifications were conducted in a total volume of either 25  $\mu$ l or 50  $\mu$ l using 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.01% gelatin, 200  $\mu$ M dNTPs, 2 mM MgCl<sub>2</sub>, 0.5 units of *Taq* polymerase (Promega Co.), 0.3  $\mu$ M of each primer, 20–150 ng of template DNA, and water to the final volume. 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 (D. L. Swofford, Sinauer Associates, Sunderland, MD, 2002) was used to calculate pairwise uncorrected *p*-distances between all haplotypes. To visually assess how haplotypes are partitioned between the species, a haplotype network was generated using TCS (Templeton et al., 2000). All sequences have been deposited in GenBank (GQ253568–GQ253573).

RESULTS

The first two axes of both PCAs accounted for less than 50% of the variance in either sex (males 32%, females 44% see Table 1). However, each species formed a distinct assemblage (Figs. 2, 3), which were interspecifically significant using MRPPs (females:  $\Delta_o = 0.1746$ ,  $\Delta_e = 0.2039$ ,  $P < 0.001$ ; males:  $\Delta_o = 0.1636$ ,  $\Delta_e = 0.1821$ ,  $P < 0.001$ ). In general, the ordinations indicated a pigmentation gradient along Axis I distinguishing the two species with *G. flavimaculata* having more yellow pigmentation and *G. oculifera* having more dark pigmentation (Figs. 2, 3). Loading scores for this axis revealed 10 variables for males and 11 variables for females, which were the most important characters differentiating the two species (Table 1). Other than the pigmentation variables, *G. oculifera* had longer anal and shorter abdominal plastral scutes. Likewise, Table 2 quantitatively summarizes the variables shown to be important by the loading scores. In both PCAs, Axis II explained approximately 11% of the variance and did not differentiate between the two species as well as Axis I (Figs. 2, 3; Table 1). Similar to the PCAs, both females and males of the two species formed distinct groups in the UPGMA analysis. However, there was not

TABLE 1. The PCA loading scores of male and female *Graptemys oculifera* and *Graptemys flavimaculata* showing several pigmentation and two plastral scute characters important in the ordination. Percentages in the parenthesis represent variance explained by axis 1 and 2, respectively.

Sex/character	Axis I	Axis II
Male (22%, 10%)		
AB	-0.22	-0.176
AN	0.212	0.116
LPOB	0.284	-0.007
WBLO	-0.252	0.042
WIOL	0.23	-0.154
WY2F	0.235	-0.214
WB24	-0.21	-0.173
WPIGD	0.293	-0.021
WVPIG	0.309	0.02
WY2H	0.241	-0.07
Female (33%, 11%)		
AB	0.215	-0.05
AN	-0.235	0.189
WPOB	-0.219	-0.051
NLU	-0.265	-0.091
NLL	-0.244	-0.133
WIOL	-0.269	-0.06
LPOB	-0.203	0.023
WY2F	-0.228	0.101
WY4F	-0.248	0.125
WPIGD	-0.246	0.151
WVPIG	-0.258	0.198

perfect separation between the two (Figs. 4, 5). Although the cophenetic correlations (females, 0.8189; males, 0.8743) indicated that the clustering moderately represented the structure in the data, the clustering in both sexes was driven by head patterns and soft tissue pigmentation (Table 3). Besides the obvious pigmentation pattern on the coastal scutes, *G. flavimaculata*

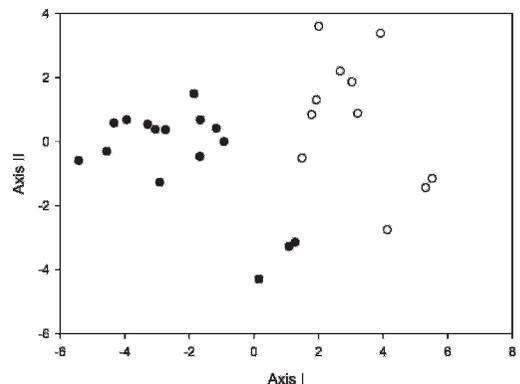


FIG. 2. A principal components analysis plot of female individuals of *Graptemys oculifera* (black circles) and *Graptemys flavimaculata* (open circles) showing a pigmentation gradient along axis I.

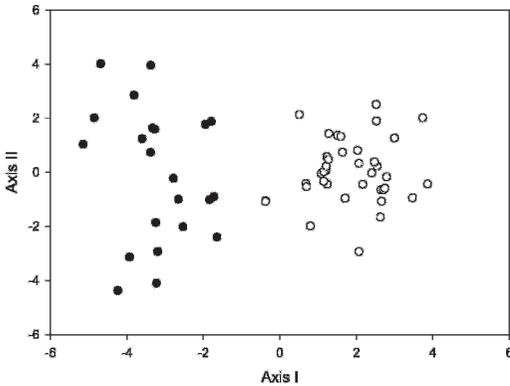


FIG. 3. A principal components analysis plot of male individuals of *Graptemys oculifera* (black circles) and *Graptemys flavimaculata* (open circles) showing a pigmentation gradient along axis I.

has more lines entering the orbit and postorbital blotch (Table 3; Fig. 6). Likewise, *G. flavimaculata* more frequently has the interorbital line extending and connecting to lateral lines at the nasal (Fig. 6), necklines that connect under the chin, and a U-shaped bar under the chin (Table 3).

We obtained six sequences per species for the CR (657 bp) and eight sequences for *G. oculifera* and six sequences for *G. flavimaculata* for the ND4 (894 bp). The CR demonstrated more variation than ND4. After combining these sequences, four unique haplotypes were found in the six individuals with data for both regions. Two haplotypes were found in each species with one more frequent than the other (Fig. 7). The two most common haplotypes were only different by two base pairs, whereas the most divergent haplotype (in *G. oculifera*) differed

from either of these haplotypes by seven base pairs.

DISCUSSION

Some of the morphological characters Cagle (1954) used to diagnose *G. flavimaculata* and *G. oculifera* actually overlapped between the species. Likewise, although our analyses of an expanded set of characters demonstrated significant morphological differentiation between the two species, some specimens occasionally had characters that overlapped with the other species. Besides the difference in costal scute markings, which is the basis for the two species' common names, *G. flavimaculata* has more yellow pigmentation on the carapace and soft tissues than does *G. oculifera*. In particular, *G. flavimaculata* has more yellow pigmentation on the first vertebral and fifth marginal scutes and has a longer postorbital blotch than *G. oculifera* (Fig. 6). Also similar to Cagle's (1954) comparison, our data showed that *G. flavimaculata* usually had yellow, dorsal necklines connecting to the postorbital blotches and a broader yellow interorbital line than did *G. oculifera* (Fig. 6).

Distinct morphologies may not always reflect strong genetic differentiation between species. Morphological differentiation may be the product of strong selection pressure, lineage sorting of polymorphism in the ancestral population, or genotype by environment interactions (Futuyma, 1998; Greenberg et al., 1998; Avise, 2000). Interestingly, head patterns, which are diagnostic traits used in *Graptemys* taxonomy (Lovich and McCoy, 1992; Vogt, 1993 and references therein), are known to be under environmental control and exhibit clinal variation in some *Graptemys* species (Ewert, 1979;

TABLE 2. Mean ratios and standard deviation (SD) of several important pigmentation characters determined by the PCA loading scores that can differentiate between *Graptemys flavimaculata* and *Graptemys oculifera* in the Pascagoula and Pearl drainages.

Species/sex	Characters													
	AB	AN	LPOB	WPOB	NLU	NLL	WBLO	WIOL	WY2F	WY4F	WB24	WPIGD	WVPIG	WY2H
<i>G. flavimaculata</i>														
Female														
Mean	0.208	0.200	0.041	0.043	0.009	0.016	0.013	0.016	0.022	0.019	0.034	0.024	0.039	0.018
SD	0.054	0.010	0.008	0.009	0.002	0.003	0.003	0.003	0.006	0.003	0.005	0.007	0.011	0.004
Male														
Mean	0.209	0.204	0.041	0.049	0.104	0.150	0.204	0.018	0.023	0.020	0.032	0.024	0.038	0.022
SD	0.033	0.012	0.006	0.009	0.020	0.033	0.045	0.004	0.005	0.003	0.005	0.008	0.011	0.004
<i>G. oculifera</i>														
Female														
Mean	0.251	0.174	0.031	0.034	0.007	0.013	0.015	0.010	0.016	0.014	0.037	0.013	0.013	0.015
SD	0.019	0.012	0.009	0.010	0.002	0.003	0.003	0.004	0.004	0.003	0.004	0.004	0.007	0.004
Male														
Mean	0.238	0.179	0.030	0.042	0.100	0.143	0.232	0.013	0.019	0.017	0.039	0.013	0.015	0.017
SD	0.017	0.037	0.008	0.012	0.011	0.016	0.023	0.005	0.005	0.005	0.008	0.004	0.006	0.003

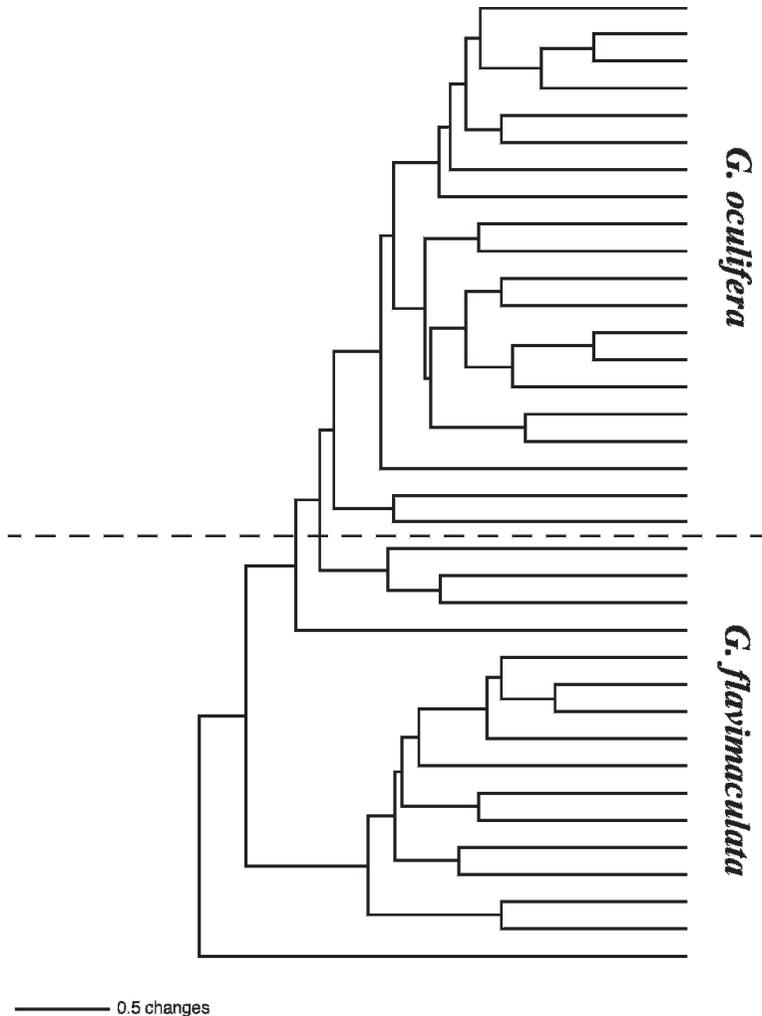


FIG. 4. The UPGMA dendrogram showing female *Graptemys oculifera* and *Graptemys flavimaculata* are diagnosable using the selected qualitative characters. The cophenetic correlation (i.e., 0.8189) suggests that the clustering represented the structure of the raw data moderately well. Dashed line indicates the delineation between species.

Vogt, 1993). Although we found a head pattern difference between *G. flavimaculata* and *G. oculifera*, this character has never been considered critical in distinguishing the two species. More important, there are no studies suggesting that the expression of other soft and hard tissue patterns that we examined are influenced by the environment. Regardless of the cause, it is worth noting that the two groups of *Graptemys* species in these drainages demonstrate the same pattern of pigmentation differentiation. *Graptemys gibbonsi* and *G. flavimaculata* in the Pascagoula both have more yellow pigmentation on the carapace relative to *G. pearlensis* and *G. oculifera* inhabiting the Pearl River (Ennen et al., 2010; this study).

Despite the significant morphological differentiation between *G. flavimaculata* and *G. oculifera*, like Lamb et al. (1994), we found limited genetic differentiation. For example, the most common composite haplotypes in either species were only different by two mutational steps. The lack of strong molecular support for *G. flavimaculata* and *G. oculifera* is probably not a function of a poor choice in molecular markers. The three mitochondrial genes (i.e., control region, *cyt b*, and ND4) used in this study and by Lamb et al. (1994) are among the most commonly employed in molecular systematic studies of turtles, and they are also among the most variable at lower taxonomic levels (Fitz-Simmons and Hart, 2007). Perhaps the inability

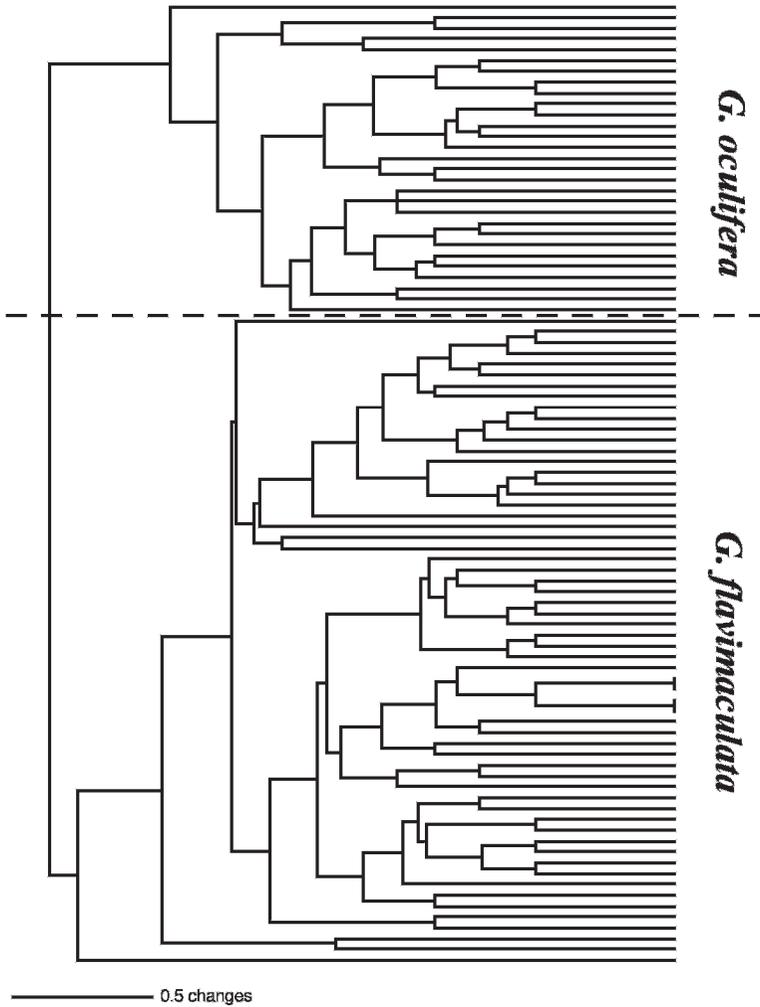


FIG. 5. The UPGMA dendrogram showing male *Graptemys oculifera* and *Graptemys flavimaculata* are diagnosable using the selected qualitative characters. The cophenetic correlation (i.e., 0.8743) suggests that the clustering represented the structure of the raw data moderately well. Dashed line indicates the delineation between species.

TABLE 3. Mean counts with standard deviation (SD) and frequencies of occurrence of several of the qualitative characters that differentiate between *Graptemys flavimaculata* and *Graptemys oculifera* in the Pascagoula and Pearl drainages.

Species/ sex	Meristics				Presence/absence						1st costal		
	#YHE	#NLPOB	#YLFE	#NLO	LLC	3YFE	NLIOL	DLPOB	NASAL	YUC	Blotch	Ring	Broken ring
<i>G. flavimaculata</i>													
F	3.0 (1.2)	1.1 (0.97)	3.4 (1.3)	3.8 (0.38)	0.88	0.71	0.84	0.59	0.63	0.94	0.87	0.08	0.05
M	3.3 (1.1)	1.8 (0.47)	3.3 (1.0)	3.1 (0.87)	0.93	0.57	0.85	0.96	0.85	0.93	0.92	0.08	0.00
<i>G. oculifera</i>													
F	2.1 (0.51)	0.17 (0.48)	2.8 (1.6)	2.5 (0.59)	0.125	0.43	0.63	0.13	0.50	0.71	0.00	0.71	0.29
M	2.2 (0.65)	0.93 (0.89)	2.1 (0.81)	2.1 (0.44)	0.30	0.13	0.58	0.58	0.58	0.52	0.00	0.89	0.11

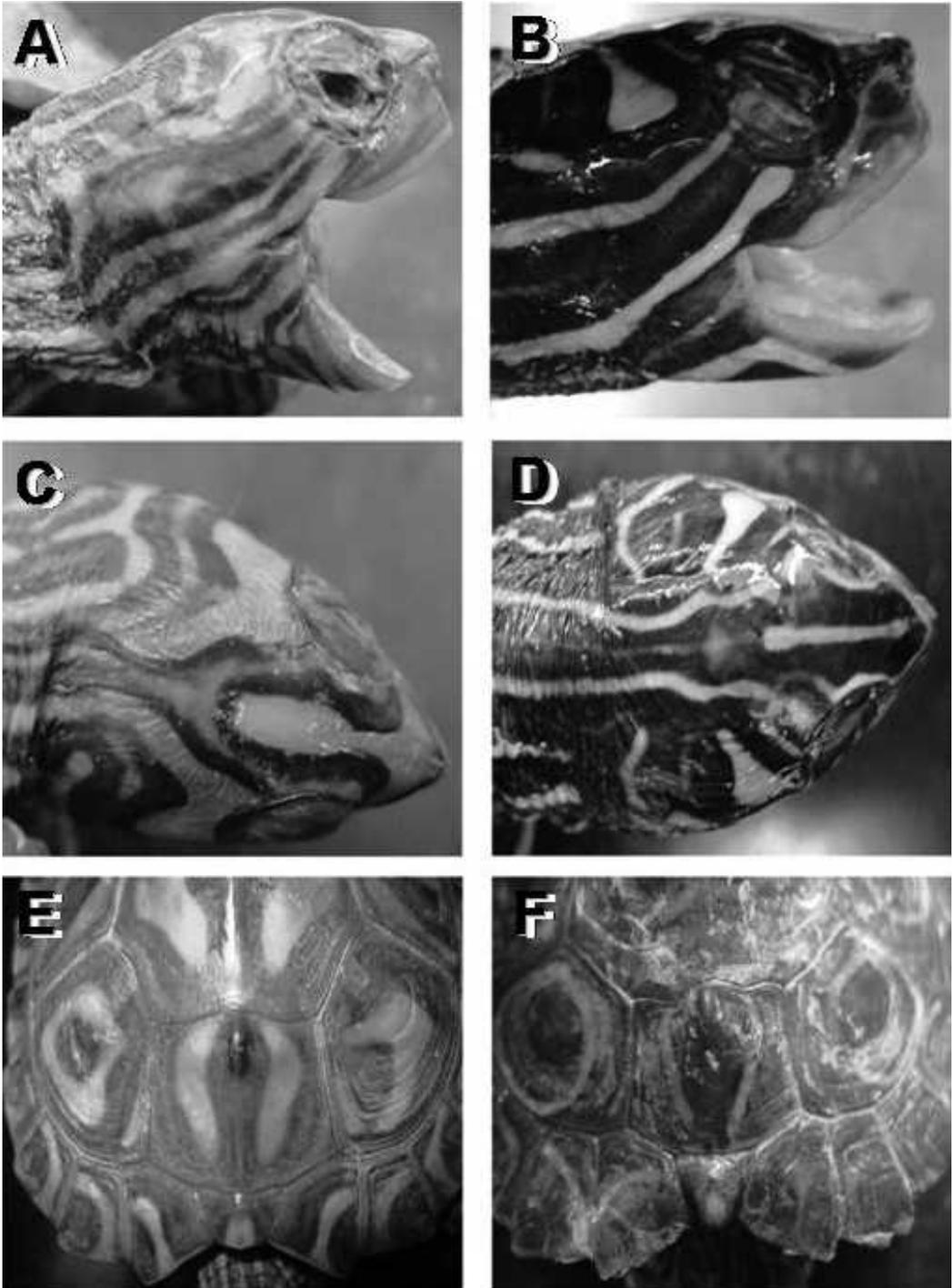


FIG. 6. Images of several adult *Graptemys flavimaculata* (left column; MMNS 5696, 1045) and *Graptemys oculifera* (right column; MMNS 8393, 3752) illustrating differences at several characters measured for the morphological analyses. (A and B) *Graptemys flavimaculata* frequently has more lines entering the orbit than *G. oculifera*. (C and D) *Graptemys oculifera* commonly has less yellow pigmentation on the head in particular length and width of postorbital blotch (LPOB and WPOB), width of interorbital line (WIOL), and the absence of interorbital line connecting at the nasal with lateral lines. (E and F) *Graptemys flavimaculata* has more yellow pigmentation on the vertebral scutes than *G. oculifera* (WVPIG).

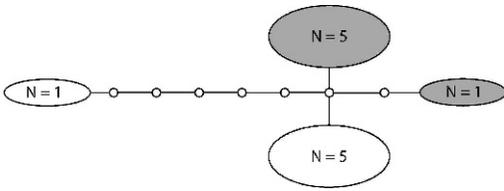


FIG. 7. The TCS generated haplotype network for the combined mtDNA sequence data. Haplotypes shaded grey represent *Graptemys flavimaculata* and white represent *Graptemys oculifera*.

of mtDNA to fully resolve the taxonomic relationships within *Graptemys* might be caused by slow evolutionary rates in chelonian mitochondrial DNA (mtDNA; Avise et al., 1992; Lamb et al., 1994). Support for this idea was recently presented by Wiens et al. (2010) who reported limited levels of variation in mtDNA within *Graptemys* (and *Pseudemys*) compared to other species in the family Emydidae. Admittedly, the limited mitochondrial divergence between species could also be a product of a complicated evolutionary history such as recent isolation of populations in the two drainages or reflect ancient hybridization between the species (e.g., Spinks and Shaffer, 2009).

Regardless of the rate of mitochondrial evolution in chelonians, some species (e.g., *Sternotherus minor*, *Sternotherus odoratus*, and *Kinosternon subrubrum*) demonstrate greater intraspecific divergence than is seen between many species of *Graptemys* (Walker and Avise, 1998), even in species such as *S. odoratus* that exhibit morphological homogeneity across its range (Reynolds and Seidel, 1983). Even within the emydids, Wiens et al. (2010) found higher levels of genetic differentiation in mitochondrial and nuclear genes among *Trachemys scripta* ssp. than between closely related species of *Graptemys*. The question remains as to whether the genus *Graptemys* may be oversplit (Walker and Avise, 1998) or whether these are valid species that are the product of recent radiations associated with periodic sea level fluctuations along the Gulf of Mexico (Wood, 1977; Lovich and McCoy, 1992).

These questions about taxonomy and evolutionary history are not strictly of academic interest. *Graptemys oculifera* and *G. flavimaculata* are both federally listed as Threatened (U.S. Fish and Wildlife Service, 1986, 1991) and listed as Endangered by the state of Mississippi (Mississippi Department of Wildlife, Fisheries, and Parks, 2000); thus, their taxonomic status has important conservation implications. This study found the two species to be morphologically distinct in a variety of pigmentation

characters, which may or may not be environmentally influenced, and hard characters such as plastral scute length. The lack of accompanying strong genetic differentiation is not necessarily surprising if these species are only recently diverged (Greenberg et al., 1998). In these situations, a more productive way to delimit species may be to take a population genetic approach by defining a species as a genetically and demographically connected metapopulation rather than a genealogical one that only recognizes monophyletic groups (Shaffer and Thomson, 2007). However, a phylogenetic approach to resolving species within *Graptemys* is not necessarily a lost cause. The work of Wiens et al. (2010) with sequence data from six nuclear loci did find that *G. flavimaculata* and *G. oculifera*, and other closely related species, were clearly genetically distinct. Thus, relying on mtDNA alone would fail to recognize potentially valid species.

The discrepancy between the morphological and mtDNA aspects of this study highlights the fact that the taxonomic status of *G. oculifera* and *G. flavimaculata* has been problematic since the description of *G. flavimaculata*. For example, in the species description, Cagle (1954) proposed an alternative taxonomy of *G. flavimaculata* that included it as a subspecies of *G. oculifera*. However, our morphological analyses clearly distinguish the two species as does the recent molecular work of Wiens et al. (2010). Thus, the combination of these two lines of evidence seems to support the continued recognition of *G. oculifera* and *G. flavimaculata* as valid species.

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## APPENDIX 1

*Specimens Examined*

*Graptemys oculifera* (N = 55).—Mississippi: Hinds County, Pearl River, MMNS 4005; Lawrence County, Pearl River, MMNS 3995, TU 14867, 21438–21450, 21645–21647, 21726–21733, 21733–21736, 21827; Leake County, Pearl River, MMNS 7681–7684, TU 21816; Madison County, Pearl River, MMNS 5639, 5640; Marion County: Pearl River, MMNS 3280–3281, 3731–3733, 3752–3756, 4023, 7686; Neshoba County, Pearl

River, MMNS 3874; Rankin County, Pearl River, MMNS 4000–4003, 8393, 15816; St. Tammany Parish, Pearl River, TU 29769; Washington Parish, Pearl River, TU 21885.

*Graptemys flavimaculata* ( $N = 93$ ).—Mississippi: Clarke County, Chickasawhay River, MMNS 10754; Covington County, Leaf River, MMNS 1026; Forrest County, Leaf River, MMNS 1057; Greene County, Chickasawhay River, MMNS 1030, 5696–5699; George County, Pascagoula River, MMNS 1039, 1040, 1043, 1045, 1052–1054, 1073–1075, 1077, 1087–1093, 1122,

4014, 4015, TU 14752, 14756–14760, 14762–14766, 14774, 14776, 14779–14785, 14799, 14804, 14806–14809, 14812, 14818, 14821, 14822, 14829, 14832, 14845, 14850, 14857, 14858, 14862, 14865, 14866, 148665, 14868–14871, 14873, 14873, 149221, 16546.1, 16546.3; Jackson County, Pascagoula River, MMNS 1066, 1105, 1114, 1117, 5641; Jones County, Eastabuchie River, MMNS 3728, MMNS 4012; Perry County, Tallahala Creek, MMNS 1022, 1023; 1072, 1081, 1082, 1121; No specific locality, MMNS 1096; No museum voucher number or specific locality, TU.