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INTERDRAINAGE MORPHOLOGICAL AND GENETIC DIFFERENTIATION IN THE ESCAMBIA MAP TURTLE, *GRAPTEMYS ERNSTI*

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***Abstract.*—*Graptemys ernsti*, the Escambia Map Turtle, inhabits the Escambia/Conecuh River, the adjacent Yellow River, and the Pea River further to the east, all of which have been distinct drainage systems since the Pleistocene. We used continuous and meristic morphological and genetic data to compare populations of *G. ernsti* and found evidence of differences among the three drainages. Frequency of occurrence of a nasal trident differed among the three drainages. Yellow River specimens possessed unique mitochondrial haplotypes while the Conecuh and the Pea shared haplotypes. Five microsatellite loci identified the drainages as being distinct, with the strongest differentiation between the Yellow River and the other two drainages. While these differences do not appear great enough to warrant taxonomic recognition, they do suggest that each population has a distinct evolutionary and demographic history and that they should therefore be managed separately.**

Key Words.—conservation; Emydidae; freshwater turtles; interdrainage variation; morphology; population genetics

INTRODUCTION

Geographic variation in natural populations has long been recognized as important to understanding the process of speciation (Gould and Johnston 1972). Variation across the range of a species can take the form of differences in morphology (Endler 1986), physiology (Feder et al. 1987) and molecular markers (Avise 2000). Natural selection can play a strong role in shaping geographic patterns of variation (Endler 1986). Ultimately, however, the maintenance of geographic variation is dependent upon the interplay between the homogenizing influence of gene flow and the evolutionary forces of mutation, drift, and natural selection, which lead to genetic differentiation (Slatkin 1987).

Genetic variation within a species can have important conservation implications, as genetically distinct populations can contribute to the overall evolutionary potential of species and represent an important part of biodiversity and ecosystem function (Hughes et al. 1997; Luck et al. 2003). In the USA, this has been formally recognized under the Endangered Species Act through the potential to designate distinct population segments (DPSs; USFWS and NOAA 1996). Distinct population segments are often equated with evolutionarily significant units (ESUs), although how these are defined is not agreed upon (Allendorf and Luikart 2007). Some ESU concepts focus on reciprocal monophyly (Moritz 1994), while others look for evidence of the lack of ecological and genetic exchangeability among populations (Crandall et al. 2000). Below the level of ESU, but still important in terms of conservation, are management units (MU's), populations with divergent allele frequencies (Moritz 1994) that are deemed to be demographically independent.

The turtle genus *Graptemys* is an example of how important, and sometimes difficult, it can be to define the appropriate units of conservation, whether at the level of species or populations. *Graptemys* is the most diverse turtle genus in North American with nine of the 14 species endemic to single rivers in the southeastern United States (Lindeman 2013). The evolution of *Graptemys* is hypothesized to be linked to glacial cycles during the Late Pliocene through the Pleistocene (Lovich and McCoy 1992; Lamb et al. 1994; Ennen et al. 2010b) and their associated sea-level fluctuations, which created morphological and genetic variation among drainages through repeated dispersal and vicariance events. However, the taxonomy and biodiversity of this group is still being explored, as demonstrated by the recent description of a new species (Ennen et al. 2010b), the

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Figure 1. Male (top) and female (bottom) *Graptemys ernsti* from the Yellow River and Conecuh River, respectively. (Photographed by James Godwin).

elevation of a subspecies to species (*G. sabinensis*; see Lindeman 2013), and assessments of the validity of other species (Ennen et al. 2010a) and subspecies (Ennen et al. 2014) designations. Past or ongoing studies have also focused on the management implications of potential population structure within species such as *G. flavimaculata* (Selman 2012; Selman et al. 2013), *G. caglei* (Ward et al. 2013), *G. oculifera* (Gaillard et al. 2015) and *G. sabinensis* (Cybil Covic Huntzinger et al., unpubl. data).

The Escambia Map Turtle, *Graptemys ernsti* (Fig. 1), inhabits three distinct river drainages (Escambia/ Conecuh, Yellow, and Pea) along the northern Gulf of Mexico (Fig. 2), but the majority of individuals inhabit the Escambia/Conecuh and Yellow rivers. The Pea population was recently discovered and there is evidence of hybridization with the sympatric Barbour's Map Turtle, *Graptemys barbouri* (Godwin et al. 2014). These populations were thought to be the result of anthropogenic translocations (Jackson 2005), but recent work suggests that *G. ernsti* entered this drainage through natural processes (Godwin et al. 2014). Regardless of the origin of the Pea populations, the dynamic nature of the geologic history of these drainages during the Pliocene and Pleistocene (Price and

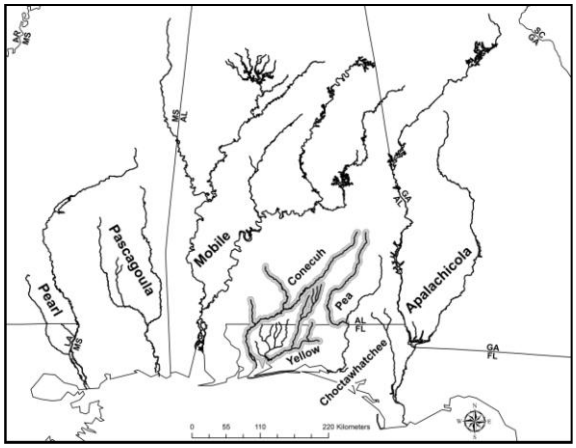

FIGURE 2. A distribution map for *Graptemys ernsti* in Alabama, USA, showing the three drainages (Escambia/Conecuh, Yellow, and Pea rivers) highlighted in gray shading. The highlighted drainages from left to right are Escambia/Conecuh River, Yellow River, and Pea rivers.

Whetstone 1977; Locker and Doyle 1992) has likely influenced the evolutionary history of *G. ernsti* across its distribution. We examined morphological and genetic variation among the Conecuh/Escambia, Yellow, and Pea River populations to understand the extent of historical connectivity, provide insight into the evolutionary history of the species, and help inform future conservation efforts. We investigated geographic variation among the three populations using six pigmentation variables, eight shell measurements, mitochondrial DNA (control region and ND4) and microsatellite markers.

MATERIALS AND METHODS

We measured live and preserved specimens of *Graptemys ernsti* (Auburn University Museum of Natural History [AUM], Florida Museum of Natural History [FLMNH], and Tulane University Museum of Natural History [TU]; Appendix 1). For the live specimens, we used the same individuals and tissues collected from Godwin et al. (2014). Because there is potential admixture between *G. ernsti* and *G. barbouri* in the Pea River, we used only individuals that Godwin et al. (2014) identified in their STRUCTURE analysis as having membership coefficients (q) for *G. ernsti* > 0.92. After removing individuals with missing morphological and/or locality data, our final morphological datasets included 54 specimens (overall: females, n = 37; males, n = 17; and by population: Yellow River, n = 17; Conecuh/Escambia River, n = 37) for the continuous variables. For our morphological dataset of meristic variables, our sample size varied for each pigmentation variable depending on missing data (Table 1); however,

TABLE 1. Percentage of *Graptemys ernsti* specimens measured from the Conecuh, Yellow, and Pea rivers exhibiting each of the pigmentation variables. Numbers within the parentheses represent sample sizes.

Variables	Conecuh River	Yellow River	Pea River
Nasal trident	100% (112)	97.6% (41)	75% (8)
POB-IOB	10% (140)	22.9% (35)	0% (8)
SUBOC	35.4% (144)	47.2% (36)	12.5% (8)

we measured 210 specimens (females, n = 68; males, n = 43; juveniles, n = 99) for our meristic variables. We measured three pigmentation characters and three pattern variables on the right side of each individual. All the pigmentation variables were selected from Lovich and McCoy (1992) and included length of postorbital blotch (LPOB), width of yellow pigmentation on the dorsal surface of the fifth marginal scute (MPIG), width of dark pigmentation on the ventral surface of the fifth marginal scute (WL5MP), presence/absence of nasal trident, presence/absence of connection between postorbital blotch and interorbital blotch (POB-IOB), and presence/absence of a subocular spot (SUBOC). Additionally, we collected straight-line measurements (mm) for maximum carapace length (CL), carapace width (CW), carapace height (CH), maximum plastron length (PL), and central seam lengths of the six plastral scutes (gular [G], humeral [H], pectoral [P], abdominal [AB], femoral [F], and anal [AN]). To account for allometry, we divided LPOB by CL, central seam lengths were divided by PL, and fifth marginal scute pigmentation widths were divided by length of the fifth marginal scute. JEL collected all continuous data and we transformed measurements using arcsine square-root. We analyzed the sexes separately to account for sexual dimorphism (Gibbons and Lovich 1990). We excluded individuals from the Pea River in the analyses of our continuous data because they were not measured by JEL. If included, these individuals could potentially introduce measurement error and confound our results (see Eason et al. 1996). We analyzed males, females, and juveniles together with our meristic datasets, which included meristic data collected by three of the authors (JRE, JG, and JEL).

Because Ennen et al. (2014) found that pigmentation and morphology appears to be influenced by cumulative drainage area (CDA; a surrogate for local stream size and hydrology) within *Graptemys*, we calculated CDA for each specimen using ArcMap 10.2.2. We used the Watershed Service of ESRI, which is a Geoprocessing Service available through ArcGIS Online (http://hydro.arcgis.com/arcgis/services). This service uses two datasets (30m National Hydrology Plus Database [NHDPlusV2.1] and 90m Hydrological Data and Maps Based on Shuttle Elevation Derivatives at Multiple Scale [HydroSHEDS]) to calculate CDA.

To investigate morphological and pigmentation differences between the Escambia/Conecuh and Yellow rivers populations, we used nonparametric multivariate analyses of variance (NP-MANOVA, adonis functions in the vegan package, Oksanen et al. 2013) and likelihood test (likelihood.test function in Deducer package; Fellows 2012) in the statistical program R (R Development Core Team, 2014). For the continuous variables, we used principal components analyses (PCA; prcomp function in R) to identify important variables (i.e., loading scores) driving population differences, to visualize the data in multidimensional space, and to obtain axes scores. Using PCA axes 1–3 scores as our dependent variables, we conducted full factorial NP-MANOVAs with drainages as our fixed effect and CDA as a covariate. We conducted all NP-MANOVAs on Euclidean distance dissimilarity matrices and permutated 10,000 times. For the meristic data, we ran individual contingency tables using a G statistic for each variable and we included all three populations in the analyses. We used an alpha of 0.05.

Excluding *G. barbouri* data, we reanalyzed data collected from a previous study on *G. ernsti* and *G. barbouri* (Godwin et al. 2014) to examine the extent of genetic differentiation among individuals of *G. ernsti* from the Escambia/Conecuh (n = 34), Yellow (n = 26) and Pea (n = 8) rivers. We used six microsatellite loci, one of which (*TerpSH*2) was monomorphic and was excluded from further analyses. We conducted tests for Hardy-Weinberg equilibrium and linkage disequilibrium using GENEPOP for the web (Raymond and Rousset 1995; http://genepop.curtin.edu.au/). We used sequential Bonferroni correction to adjust our alpha levels for these two tests (Rice 1989), and we measured genetic variation within each drainage by the observed heterozygosity (H_o) and expected heterozygosity (H_e) as calculated by GenAlEx 6.501 (Peakall and Smouse 2006) and allelic richness (A_R) as calculated by FSTAT 2.9.3 (Goudet 1995). FSTAT 2.9.3 was also used to calculate Weir and Cockerham's (1984) unbiased estimator of F_{ST} and perform significance testing of this value. We also employed the Bayesian approach used by STRUCTURE 2.3.3 (Pritchard et al. 2000) to determine the number of genetically discrete populations (K) represented by the data. We used sample location as a prior (Hubisz et al. 2009) and correlated allele frequencies were assumed with potential admixture between groups. We tested values of K from 1–6 and for each value of K we performed 20 independent runs with a burn-in of 250,000 followed by a subsequent 500,000 Markov Chain Monte Carlo replications. We selected the best value of K based on the probability scores and the ΔK analysis (Evanno et al. 2005) as calculated by Structure Harvester v 6.92 (Earl and von Holdt 2012). We

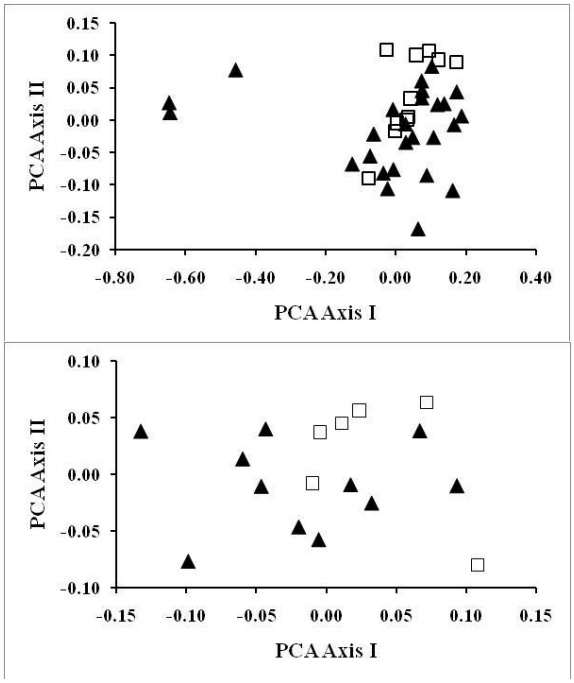

FIGURE 3. Principal component analysis plots of morphological data for *Graptemys ernsti* females (top) and males (bottom) from the Conecuh River (solid triangles) and Yellow River (open squares) in Alabama, USA.

averaged the results for a given value of K with CLUMPP v. 1.1.2 (Jakobsson and Rosenberg 2007) and then visualized with DISTRUCT v. 1.1 (Rosenberg 2004).

Furthermore, we sequenced portions of the mitochondrial control region and NADH dehydrogenase subunit 4 (ND4) using the methods described by Ennen et al. (2010a) for representative individuals from the Conecuh (n = 3), Yellow (n = 6), and Pea (n = 7) rivers. We also included previously published sequences of two *G. ernsti* from the Conecuh River (GQ856231 and GQ856220–21; Appendix 2). We edited and aligned the sequence data using Sequencher v. 4.10.1 (GeneCodes Co., Ann Arbor, Michigan, USA) and concatenated the control region and ND4 sequences for each individual prior to analysis. We used PAUP* 4.0b10 (Swofford 2002) to calculate pairwise uncorrected *p* distances between all haplotypes and a haplotype network was generated using TCS v. 1.21 (Clement et al. 2000).

RESULTS

Morphometrics.—Using our continuous dataset, the first three PCA axes accounted for 86% of the variance for males and 94% of the variation for females. Plots of the first two PCA axes described the Yellow and Conecuh River populations as overlap groups in both sexes (Fig. 3). In females, variation in the Conecuh River was greater than in the Yellow River, with females

TABLE 2. Loading scores from the principal component analyses for female and male *Graptemys ernsti*. Percentages are the amount of variation explained by each axis. Acronyms are carapace width (CW), carapace height (CH), plastral gular (G), plastral humeral (H), plastral pectoral (P), plastral abdominal (AB), plastral femoral (F), plastral anal (AN), length of postorbital blotch (LPOB), width of yellow pigmentation on the dorsal surface of the fifth marginal scute (MPIG), and width of dark pigmentation on the ventral surface of the fifth marginal scute (WL5MP).

	PCA Axis		
Sex/Variable	I	II	III
Female	81.2%	10.0%	3.2%
CW	0.06	0.50	-0.59
CH	0.00	0.09	-0.29
G	0.05	0.11	0.22
H	-0.07	-0.20	-0.23
P	0.08	0.26	-0.03
AB	-0.03	-0.11	-0.05
F	-0.02	-0.21	-0.20
AN	0.04	0.32	0.02
LPOB	-0.05	-0.04	0.54
MPIG	0.07	0.66	0.34
WL5MP	-0.99	0.15	0.00
Male	48.9%	24.6%	12.4%
CW	0.35	0.50	0.61
CH	0.15	0.17	0.19
G	0.14	0.10	-0.12
H	-0.19	-0.06	-0.03
P	0.11	0.15	0.21
AB	0.07	0.07	0.13
F	-0.13	-0.11	-0.07
AN	0.08	0.17	-0.02
LPOB	0.02	0.09	-0.10
MPIG	0.36	0.53	-0.71
WL5MP	0.79	-0.59	-0.02

of the latter occupying a subset of the variation of the former in morphospace, while variation of males overlapped less and showed greater interdrainage differentiation in morphospace. The first PCA axis separated the male specimens from the two populations along a pigmentation gradient based mostly on WL5MP (Tables 2 and 3). This separation was only marginally insignificant ($F_{1,16}$ = 2.67, P = 0.068), while female pigmentation and morphology were not different between the Yellow and Conecuh rivers ($F_{1,36}$ = 1.21, P = 0.307). CDA was not significant in the male ($F_{1,16}$ = 2.44, P = 0.150) or female analysis ($F_{1,36}$ = 0.21, P= 0.694). There was no significant interaction effect between drainage and CDA for females ($F_{1,36}$ = 0.66, P = 0.473) or males ($F_{1,16}$ = 0.86, P = 0.463).

Frequency of occurrence for the nasal trident pattern differed among the drainages (Conecuh - 100%, Yellow - 97.6%, and Pea - 75%; G = 11.44, df = 2, P = 0.003). The SUBOC pattern displayed some variation among the river populations (Conecuh - 35%, Yellow - 47%, and Pea - 13%) but was not significantly different (G = 4.14, df = 2, P = 0.126). The frequency of occurrence for the POB-IOB pattern was not significantly different (G =

TABLE 3. Mean (standard deviation) of eight shell measurements and three pigmentation variables from preserved and live specimens of *Graptemys ernsti* from the Conecuh and Yellow rivers. Acronyms are carapace width (CW), carapace height (CH), plastral gular (G), plastral humeral (H), plastral pectoral (P), plastral abdominal (AB), plastral femoral (F), plastral anal (AN), length of postorbital blotch (LPOB), width of yellow pigmentation on the dorsal surface of the fifth marginal scute (MPIG), and width of dark pigmentation on the ventral surface of the fifth marginal scute (WL5MP).

Sex/Drainage	CW	CH	G	H	P	AB	F	AN	LPOB	MPIG	WL5MP
Conecuh River											
Female	1.05	0.72	0.36	0.32	0.37	0.52	0.42	0.47	0.33	0.37	0.95
	(0.04)	(0.02)	(0.02)	(0.03)	(0.03)	(0.02)	(0.02)	(0.03)	(0.04)	(0.04)	(0.22)
Male	1.06	0.71	0.33	0.32	0.37	0.5	0.42	0.48	0.29	0.38	0.85
	(0.05)	(0.02)	(0.01)	(0.02)	(0.02)	(0.02)	(0.01)	(0.01)	(0.01)	(0.03)	(0.06)
Yellow River											
Female	1.07	0.72	0.37	0.3	0.39	0.51	0.4	0.48	0.34	0.42	0.90
	(0.04)	(0.02)	(0.01)	(0.02)	(0.02)	(0.01)	(0.01)	(0.02)	(0.02)	(0.06)	(0.06)
Male	1.08	0.72	0.34	0.31	0.38	0.51	0.41	0.49	0.31	0.43	0.88
	(0.02)	(0.01)	(0.02)	(0.02)	(0.02)	(0.01)	(0.01)	(0.01)	(0.02)	(0.04)	(0.06)

5.80, df = 2, P = 0.055) among the rivers (Conecuh - 10%, Yellow - 23%, and Pea 0%).

Genetic results.—None of the loci deviated from Hardy-Weinberg equilibrium expectations nor demonstrated linkage disequilibrium after sequential Bonferroni correction. In terms of all measures of genetic variation, the Conecuh population was most variable, followed by the Pea and then the Yellow populations (Table 4). All pairwise F_{ST} values were significantly different from zero with a high of 0.301 for Yellow/Pea, 0.127 for Conecuh/Yellow, and 0.110 for Conecuh/Pea comparisons. The ΔK analysis of the STRUCTURE results supported the recognition of two genetic groups. However, the highest average likelihood was for K = 3 (average lnL = -880.8; SD = 0.54) rather than K = 2 (average lnL = -896.2; SD = 0.89). For K = 2, individuals from the Yellow and Pea rivers had very high membership coefficients (q scores) in one of the two groups with average q scores (± SD) of 0.985 (± 0.01) and 0.984 (± 0.01), respectively (Fig. 3). Individuals from the Conecuh River had the greatest amount of ancestry in the group represented by the Pea River with an average q score of 0.802 (± 0.04). At K = 3, each of the genetic groups represented one of the three drainages (Fig. 4).

Individuals from the Yellow River still had high membership coefficients in their own group (average q of 0.975 ± 0.01). However, membership scores for individuals from the Conecuh and Pea rivers were somewhat lower for their respective groups with average values of 0.878 (± 0.02) and 0.841 (± 0.01), respectively. For the 894-base pair (bp) ND4 region, there were two unique haplotypes, while the control region (659–660 bp) was characterized by six unique haplotypes. Similarly, for the combined ND4 and control region sequences, there were six unique haplotypes that differed from one another by between one and six base substitutions or indels (0.1–0.4% uncorrected p distance). Samples from the Conecuh and Pea rivers shared two of the three haplotypes. The remaining three

TABLE 4. Genetic variation of three populations of *Graptemys ernsti* based on six microsatellite loci. A_R represents allelic richness, H_o represents observed heterozygosity, and H_e represents expected heterozygosity.

Drainage	A_R	H_o	H_e
Conecuh	4.55	0.741	0.715
Yellow	3.00	0.475	0.456
Pea	4.52	0.639	0.608

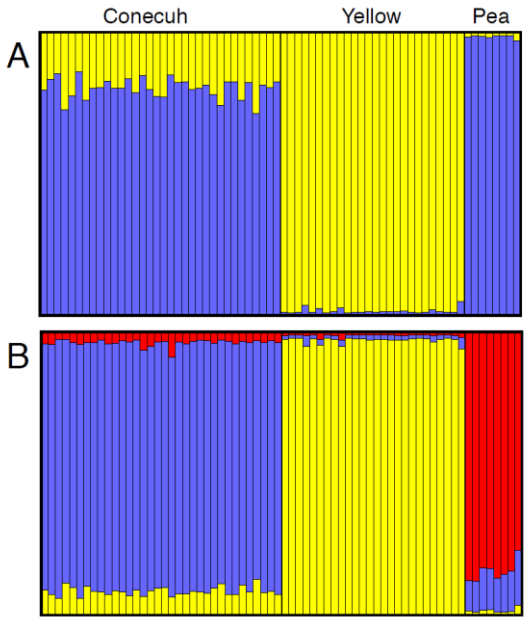

FIGURE 4. Bar plots of membership coefficients for (A) K=2 and (B) K=3 from the STRUCTURE analysis of *Graptemys ernsti* from the Conecuh, Yellow and Pea rivers in Alabama, USA.

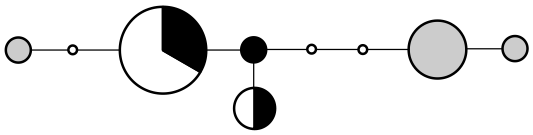

FIGURE 5. Haplotype network for the concatenated mitochondrial control region and ND4 sequences for *Graptemys ernsti* from the Conecuh (black), Yellow (gray), and Pea rivers (white) in Alabama, USA. The size of the haplotype indicates its frequency with values of nine, four, two, and one being represented. The smallest open circles represent mutational steps between haplotypes.

haplotypes were unique to the Yellow River, although these haplotypes did not entirely group together within the haplotype network (Fig. 5). We deposited all sequences in GenBank (Appendix 2).

DISCUSSION

We found significant morphological differences among the Escambia/Conecuh, Yellow, and Pea river populations of *Graptemys ernsti*. Morphologically, the populations differed by frequency of occurrence for the nasal trident and were marginally different by frequency of occurrence for the POB-IOB pattern. Additionally, male specimens were marginally different between the Yellow and Escambia/Conecuh river populations in our continuous dataset, and this marginal difference was driven by the WL5MP variable. All these pigmentation variables are important variables for distinguishing among several *Graptemys* species inhabiting coastal drainages of the northern Gulf of Mexico especially within the *pulchra* clade of the *Graptemys* genus. For example, nasal trident and WL5MP were variables used to split *Graptemys pulchra* into three distinctive species, which included *G. ernsti* and *Graptemys gibbonsi* (*sensu lato*; Lovich and McCoy 1992). Ennen et al. (2010b) found POB-IOB and SUBOC not statistically significant in discriminating between *G. pearlensis* and *G. gibbonsi* and that the nasal trident variable approached significance between the species.

Our molecular analyses found some degree of genetic differences among populations. For the microsatellite data, all pairwise F_{ST} values were significantly different from zero with the largest value between the Yellow and the Pea river populations and the smallest between the Escambia/Conecuh and the Pea river populations. This pattern is also seen in the STRUCTURE results. At K = 2, individuals from the Yellow and Pea rivers were clearly distinct, and similarly, turtles from each drainage comprised their own genetic groups at K = 3. The mtDNA data were mostly congruent with the microsatellite results, but there was limited divergence among the haplotypes. Turtles from the Escambia/Conecuh and the Pea rivers shared two haplotypes, and turtles from the Yellow River had their own set of three unique haplotypes, although these did not exclusively group together on one side of the small phylogenetic break (two mutational steps) in the network. In comparison to other studies, the limited degree of genetic differentiation in the mtDNA is not surprising. Our uncorrected p-distances for the control region (i.e., 0.40%) were slightly greater than the 0.15–0.30% reported for intradrainage variation within *Graptemys nigrinoda* (Ennen et al. 2014). We also compared our results to recent species-level comparisons within *Graptemys*. Our uncorrected p-distances were very similar to values reported between *Graptemys flavimaculata* and *Graptemys oculifera* (i.e., 0.5% CR and 0.1% ND4; Ennen et al. 2010a), but somewhat lower than the differences between *G. pearlensis* and *G. gibbonsi* for CR (i.e., 1.3% CR; Ennen et al. 2010b).

The fact that both *Graptemys ernsti* and *G. barbouri* had been overlooked for so long in the Choctawhatchee River system was a major point of discussion by Godwin et al. (2014), who suggested that both species had historically been present in this drainage, rather than representing recent introductions. For *G. ernsti*, this assertion was based on analysis of microsatellite data, which indicated that turtles from the Escambia/Conecuh, Yellow, and Pea rivers were genetically distinct. Geological evidence suggested that the three systems may have shared a delta during the Pliocene or Pleistocene (Locker and Doyle 1992), providing a potential route of dispersal. Alternatively, stream capture events may have taken place between drainages leading to the presence of *G. ernsti* in the Pea River. Godwin et al. (2014) pointed to capture of a portion of Lightwood Knot Creek in the Yellow river drainage by the Pea as one such possibility. However, both the mtDNA and microsatellite data presented here demonstrate that *G. ernsti* in the Pea are more genetically similar to the Escambia/Conecuh rather than the Yellow river. A stream capture event involving a portion of the upper Escambia/Conecuh and Pea rivers could explain our results. Stream capture events have long been hypothesized as a mechanism shaping the evolution and speciation of fish species in the southeastern United States (see Swift et al. 1986) but also for other *Graptemys* species as well (see Tinkle 1958; Lindeman 2013). Admittedly, the mtDNA data are somewhat ambiguous in addressing this question because the distribution of haplotypes could be an artifact of limited sampling that failed to detect shared haplotypes among all the rivers, or it could be the product of lineage sorting where haplotypes were historically more widespread. Turtles from the Yellow River stood out as being clearly genetically distinct from other populations at both K = 2 and K = 3 in the STRUCTURE analysis, which would also seem to suggest a more recent connection between the

Escambia/Conecuh and Pea Rivers. However, again, there are other explanations such as genetic drift, perhaps through a population bottleneck, acting to increase the genetic distinctiveness of turtles from the Yellow River relative to the other two rivers. Unfortunately, we do not have sufficient data in terms of the number of loci or individuals to effectively test this hypothesis.

Although there are morphological and genetic differences of *G. ernsti* among drainages, we hesitate to suggest that this is sufficient to warrant further taxonomic revision. Additional work with a more explicit phylogenetic approach using multiple loci (e.g., Wiens et al. 2010) would be needed to fully address this issue. However, we do suggest that our work has implications regarding the conservation and management of the species. Like many other species in the genus, *G. ernsti* has a relatively restricted distribution (Buhlmann et al. 2009), which is partially the reason for the global conservation status of the species as imperiled (NatureServe 2014; NatureServe Explorer: An online encyclopedia of life [web application]. Version 7.1. NatureServe, Arlington, Virginia. Available from http://explorer.natureserve.org. [Accessed 29 January 2015]) and/or near threatened (Lovich et al. 2011). While the distribution of *G. ernsti* encompasses relatively small portions of Alabama and Florida, the species has state conservation status of Special Concern in Alabama, but no specific status in Florida (Lindeman 2013). The species is now being considered by the United States Fish and Wildlife Service (USFWS) for protection under the Endangered Species Act (USFWS 2011). While the three rivers may not necessarily represent distinct ESUs, especially if criteria like reciprocal monophyly are applied, we do feel that the extent of differentiation we detected does reflect a demographic independence supporting their recognition as distinct management units. Thus, we recommend that the Escambia/Conecuh, Yellow, and Pea river populations of *G. ernsti* should be managed accordingly to ensure the protection of genetic and morphological diversity, and evolutionary potential within the species.

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APPENDIX 1. Museum and catalog numbers for the *Graptemys ernsti* specimens used in this study from Auburn University Museum of Natural History (AUM), Florida Museum of Natural History (FLMNH), and Tulane University Museum of Natural History (TU).

Museum	Catalog Numbers
AUM	3878, 5002. 5003, 5007, 5008, 5596, 5907, 6282, 6312, 8845, 10095, 10097-99, 13649-52, 13686, 18233, 19501, 21970, 21972, 21980-21987, 21989, 22017-22028, 31878-31880, 31883-85, 31890, 31900, 31902, 31904, 32453-32456, 32754-32770
FLMNH	158837, 159061,170596
TU	13446-13448, 13456, 13458, 13461, 13463, 15827.00-15827.40, 16576.00-16576.11, 16576.05, 16576.07, 16576.09-16576.11, 16580.00-16580.07, 16665.01-16665.05, 16665.07

APPENDIX 2. Sample identification (ID), sample locale NADH dehydrogenase subunit 4 (ND4), and the control region (CR) for individuals with mitochondrial sequence data used in this study. The sample locale (river system) for each specimen is listed along with the GenBank accession number for each unique sequence.

Sample ID	Sample locale	ND4	CR
G. ernsti-1	Conecuh	GQ856231	GQ856220
G. ernsti-2	Conecuh	GQ856231	GQ856221
Godwin-001	Conecuh	GQ856231	GQ856221
Godwin-002	Conecuh	GQ856231	GQ856221
Godwin-003	Conecuh	GQ856231	KP842821
Godwin-051	Yellow	KP842825	KP842822
Godwin-052	Yellow	KP842825	KP842822
Godwin-053	Yellow	KP842825	KP842823
Godwin-054	Yellow	GQ856231	KP842824
Godwin-055	Yellow	KP842825	KP842822
Godwin-056	Yellow	KP842825	KP842822
Godwin-006	Pea	GQ856231	GQ856221
Godwin-007	Pea	GQ856231	GQ856221
Godwin-016	Pea	GQ856231	GQ856221
Godwin-021	Pea	GQ856231	GQ856221
Godwin-024	Pea	GQ856231	GQ856220
Godwin-028	Pea	GQ856231	GQ856221
Godwin-032	Pea	GQ856231	GQ856221