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Hiding in Plain Sight: Federally Protected Ringed Map Turtles (*Graptemys oculifera*) Found in a New River System

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***Abstract.*—Understanding the geographical range of a species is essential to successful conservation and management, but their ranges are not always fully known. Ringed Map Turtles (*Graptemys oculifera*) have been federally listed as a Threatened species since 1986, and they have long been considered endemic to the Pearl River system of central Mississippi and southeastern Louisiana, USA. Based on a 2021 citizen scientist observation, a new *G. oculifera* population was discovered in the Bogue Falaya, a river system that is west of and isolated from the Pearl River system. Genetic analyses of 23 individuals from the Bogue Falaya demonstrate their genetic distinctiveness relative to sites in the Pearl River, suggesting it is a natural rather than introduced population. Therefore, *G. oculifera* should no longer be considered endemic to the Pearl River system, and this Bogue Falaya population of *G. oculifera* may warrant the designation of a distinct population segment under the U.S. Endangered Species Act. A thorough assessment of the distribution, abundance, and conservation threats to the Bogue Falaya population of *G. oculifera* as well as surveys of surrounding systems could help to inform future management actions. This discovery of a long-time federally protected species in the city limits of Covington, Louisiana, documents how citizen scientists can advance scientific knowledge.**

Key Words.—Bogue Falaya; distinct population segment; Emydidae; Endangered Species Act; genetic structure; species distribution; Testudines

Introduction

Determining presence and absence of a species is vital to understanding any physical and environmental limits to its range, and this is essential to successful conservation and management (Stryszowska et al. 2016; Sofaer et al. 2019). Distributional knowledge of many species, however, is often coarse or incomplete (Sofaer et al. 2019). The need to fully understand the distribution of species will have an increased urgency in the coming decades due to rapidly changing and novel conditions brought about by habitat destruction, disease, invasive species, pollution, and climate change (Parmesan et al. 2005; Bridle and Hoffman 2022). For listed species under the U.S. Endangered Species Act (ESA), information about where a species exists or could exist is a key component to legally binding decisions, such as regulatory actions that have major conservation and management consequences (Schwartz 2008).

One group of North American turtles, map turtles and sawbacks (genus *Graptemys*), has many members that are known to be limited to certain river drainages of the southeastern U.S. (Lindeman 2013). Because most *Graptemys* species are distributionally limited and face a high number of threats, many are considered Species of Greatest Conservation Need in their respective range states, such as Ringed Map Turtles (*Graptemys oculifera*) and Pearl River Map Turtles (*G. pearlensis*) in both Mississippi (Mississippi Museum of Natural Science 2015) and Louisiana (Holcomb et al. 2015). Consequently, numerous surveys have been conducted within the last 15 y on many species to determine their range-wide distribution. Consistently, these studies have found *Graptemys* species to occur in multiple rivers rather than a single drainage (e.g., Sabine Map Turtles, *G. sabinensis*: Ilgen et al. 2014; Barbour's Map Turtles, *G. barbouri*: Godwin et al. 2014), found new river/

creek systems where the species had not previously been recorded (e.g., *G. pearlensis*: Lindeman et al. 2020; Pascagoula Map Turtles, *G. gibbonsi*: Selman and Qualls 2009), or found major range extensions within known river systems (e.g., Texas Map Turtles, *G. versa*: Lindeman 2014; *G. barbouri*: Mays and Hill 2020). Thus, the ranges of many *Graptemys* species have been previously underestimated and should not be considered completely delineated.

Two of the river-drainage restricted species are *G. oculifera* and *G. pearlensis*, which are only known to inhabit the Pearl River system of central Mississippi and southeastern Louisiana. Both species occur sympatrically in the system, and both were long thought to be endemic to that single river system. *Graptemys oculifera* was federally listed by the U.S. Fish and Wildlife Service (USFWS) as a Threatened species in 1986 (USFWS 1986). It is also listed as a Threatened species and a Tier 1 Species of Greatest Conservation Need (SGCN) in Louisiana (Holcomb et al. 2015) and a Vulnerable species (VU) on the International Union for the Conservation of Nature (IUCN) Red List (van Dijk 2011). Here, we detail the discovery of *G. oculifera* outside of the Pearl River system. Our objective was to first determine if this was merely a few individuals or an established population, and if determined to be the latter, was this population the result of an anthropogenic introduction or did it represent a natural occurrence.

Materials and Methods

Initial discovery.—One of us (BG), saw a couple of intriguing images on iNaturalist on 3 August 2021 (e.g., https://www.inaturalist.org/observations/88686033) labeled only as *Graptemys*, apparently from Covington, Louisiana, USA (Fig. 1). In one photograph, two of the three turtles basking on a log appeared to be large female *G. oculifera*, whereas the second photograph showed what appeared to be an adult male *G. oculifera*. After confirming with the photographer (AF), who took the photographs on 17 June 2021, that he indeed saw these turtles in the Bogue Falaya (Choctaw words meaning long river) in Covington, BG immediately sought confirmation of his identification from WS, who agreed these were *G. oculifera*. After a trip to Bogue Falaya Wayside Park by BG and a technician on 25 August 2021, where he photographed another small *G. oculifera*, we decided to investigate the source of this *G. oculifera* population.

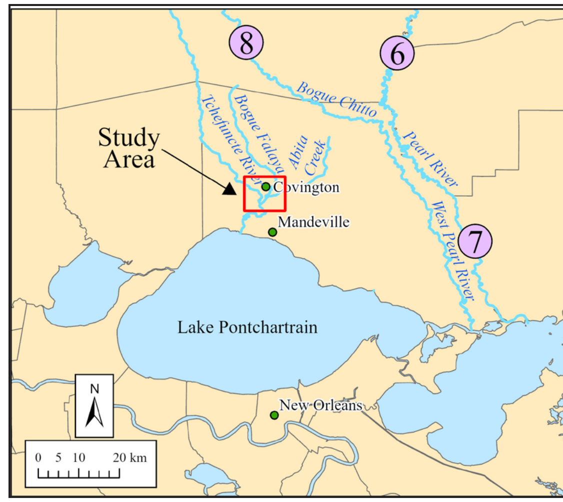

Figure 1. Location of the study area in Louisiana, USA. The red square depicts the location from where tissue samples for this study were acquired. Water bodies and city names mentioned in the manuscript are denoted. The circled numbers 6, 7, and 8 correspond to the site numbers of Gaillard et al. (2015).

Tissue collection and genetic analyses.—We made five trips to capture *G. oculifera* in the Bogue Falaya in Covington between 22 October 2021 and 9 November 2022. We captured individuals with dipnets, by hand, and with basking traps, which are standard techniques (see Selman et al. 2012). We obtained tissue samples from all captured individuals by clipping the terminal end of the tail with scissors and placing the tissue into vials of 95% ethanol. We stored vials with tail tips at room temperature in the laboratory.

We extracted genomic DNA using a DNeasy Blood & Tissue Kit (Qiagen). We amplified six microsatellite loci using the methods described in Gaillard et al. (2015). We visualized microsatellite alleles on a LI-COR 4300 DNA sequencer and we scored allele sizes scored using Gene Profiler v. 3.55 (LI-COR). We screened loci for linkage disequilibrium and deviation from Hardy-Weinberg equilibrium expectations using the R package genepop (Raymond and Rousset 1995; Rousset 2008; Rousset et al. 2020) in R version 4.2.2 (R Core Team 2022). We adjusted significance levels for multiple comparisons with a sequential Bonferroni correction (Rice 1989). For subsequent analyses, and to compare with known Pearl River populations, we included data for 229 *G. oculifera* from eight sites across the Pearl River system (Gaillard et al. 2015). We calculated metrics of genetic diversity at each site, including the number of alleles (N_A), observed heterozygosity (H_o), and expected heterozygosity (H_e), using the program GenAlEx 6.51 (Peakall and Smouse 2012). We calculated allelic richness (A_R)

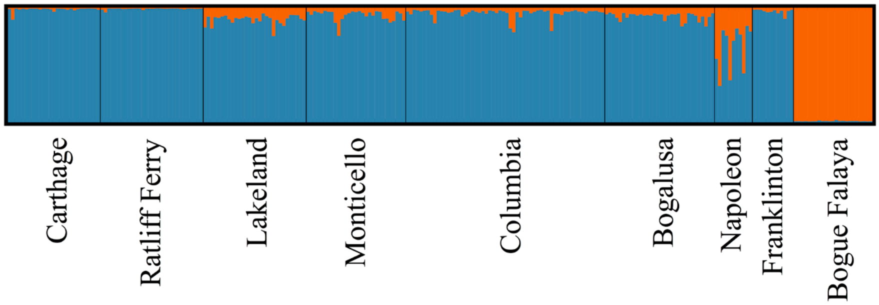

Figure 2. Bar plots of membership coefficients for Ringed Map Turtles (*Graptemys oculifera*) in the STRUCTURE analyses of microsatellite data ($K = 2$). Site names correspond to those from Gaillard et al. (2015) other than the addition of the Bogue Falaya from this study.

using the R package hierfstat (Goudet 2005). Shapiro-Wilk tests of normality were all significantly different from normal for each of the metrics, so we used the nonparametric Kruskal-Wallis Test to statistically compare the measures of genetic diversity among sites in R (R Core Team 2022).

We used STRUCTURE v. 2.3.4 (Pritchard et al. 2000) to determine the number of genetically distinct groups in the microsatellite data. This program uses a Bayesian approach to assign individuals to groups that minimizes linkage disequilibrium and deviation from Hardy-Weinberg equilibrium. We tested values of K (number of clusters) from 1–6 using a model of admixed ancestry and assuming correlated allele frequencies. We also used site location as prior information (Hubisz et al. 2009). For each value of K, we ran 20 replicates with a burn-in of 100,000 generations followed by a subsequent 500,000 generations. We determined the best value of K by comparing the average likelihood scores and by examining the ΔK values (Evanno et al. 2005) as calculated by StructureSelector (Li and Liu 2018). We also visualized patterns of differentiation among sites via a Principal Coordinates Analysis (PCoA) of unbiased genetic distances (Nei 1978) using the R package adegenet (Jombart 2008). We also measured genetic differentiation between sites by F_{ST} (Weir and Cockerham 1984) as calculated by the R package hierfstat.

In addition, we used the primers of Spinks and Shaffer (2005) to amplify a portion of the mitochondrial control region following the conditions reported by Ennen et al. (2010). We checked reactions on a 1% agarose gel stained with ethidium bromide, and we sent successful amplifications to Eurofins Scientific (Louisville, Kentucky, USA) for Sanger sequencing. We edited and aligned sequences using Sequencher v. 5.1 (GeneCodes Co., Madison, Wisconsin, USA). Our sequences were comprised of four individuals from Bogue Falaya. We also sequenced eight *G. oculifera* samples previously collected by Gaillard et al. (2015), one individual from each of the eight sites in that study. In the alignment, we also included four previously published control region sequences on GenBank from Ennen et al. (2010) comprised of two for *G. oculifera* (GenBank Accession numbers GQ253570-GQ253571) and two from the closely related *G. flavimaculata* (GenBank Accession numbers GQ253568-GQ253569). We identified unique haplotypes and examined their relationships with a minimum spanning network as constructed by the program PopART (Leigh and Bryant 2015). Lastly, we used PAUP* v. 4.0a169 (Swofford 2019) to calculate the pairwise absolute and uncorrected p distances among the unique haplotypes.

Results

Between 22 October 2021 and 9 November 2022, we captured 23 individual *G. oculifera*, including hatchlings, juveniles, and adults. We caught three hatchlings near the Bogue Falaya Wayside Park with all others downstream, extending about 3.65 river km to our southernmost capture just south of the confluence of the Bogue Falaya and Abita River. We genotyped 23 individuals from the Bogue Falaya, and none of the microsatellite loci at this site deviated from Hardy-Weinberg equilibrium or demonstrated linkage disequilibrium. Basic summary statistics of genetic diversity were calculated for the Bogue Falaya site and compared with sites from Gaillard et al. (2015;). With one exception, the Bogue Falaya site possessed lower levels of genetic diversity for each of the metrics (N_A, H_o, H_e, A_R), but the four

Table 1. The sample size (n), number of alleles (N_A), allelic richness (A_R), observed heterozygosity (H_o), and expected heterozygosity (H_e) averaged across the six microsatellite loci (standard error in parentheses below) for Ringed Map Turtles (*Graptemys oculifera*) sites. Site names, numbers, and data for sites #1–8 correspond to those from Gaillard et al. (2015), and the values for site #9 (Bogue Falaya) are from this study.

Site Name	n	N_A	A_R	H_o	H_e
Carthage (1)	27	5.0	4.0	0.503	0.560
		(1.15)	(0.78)	(0.11)	(0.07)
Ratliff Ferry (2)	30	4.7	3.9	0.585	0.566
		(0.84)	(0.65)	(0.06)	(0.06)
Lakeland (3)	30	4.5	3.8	0.564	0.552
		(0.99)	(0.61)	(0.09)	(0.07)
Monticello (4)	29	4.7	3.8	0.527	0.626
		(0.95)	(0.61)	(0.06)	(0.04)
Columbia (5)	58	4.8	3.9	0.570	0.605
		(0.95)	(0.62)	(0.10)	(0.06)
Bogalusa (6)	32	4.5	3.7	0.588	0.571
		(0.92)	(0.60)	(0.07)	(0.06)
Napoleon (7)	11	3.3	3.3	0.530	0.502
		(0.42)	(0.42)	(0.12)	(0.08)
Franklinton (8)	12	3.7	3.6	0.403	0.477
		(0.88)	(0.85)	(0.10)	(0.11)
Bogue Falaya (9)	23	2.5	2.5	0.495	0.461
		(0.34)	(0.32)	(0.13)	(0.07)

values did not differ significantly among sites (N_A, $H = 7.53$, df = 8, $P = 0.481$; H_o, $H = 3.28$, df = 8, $P = 0.916$; H_e, $H = 3.96$, df = 8, $P = 0.861$; A_R, $H = 5.40$, df = 8, $P = 0.714$).

The ΔK analysis of the STRUCTURE results for the six microsatellite loci suggested that there were two genetic groups. The log likelihood scores plateaued at $K = 3$ (Appendix Figure), but there was a large standard deviation associated with this value. We elected to interpret the results for $K = 2$, where the Bogue Falaya site represented one genetic group (mean q score = 0.99) and the other sites generally had the highest degree of ancestry in the other group (mean q scores > 0.90). The Napoleon site was the only location where individuals showed appreciable admixture between the two groups (Fig. 2, Table 2). The genetic distinctiveness of the Bogue Falaya site was also reflected in the PCoA (Fig. 3). The first axis (explaining 59.3% of the variation) separated the Bogue Falaya site from all other sites, whereas the second axis (explaining an additional 18.3% of the variation) roughly separated sites along the north-south axis of the Pearl River. The Bogue

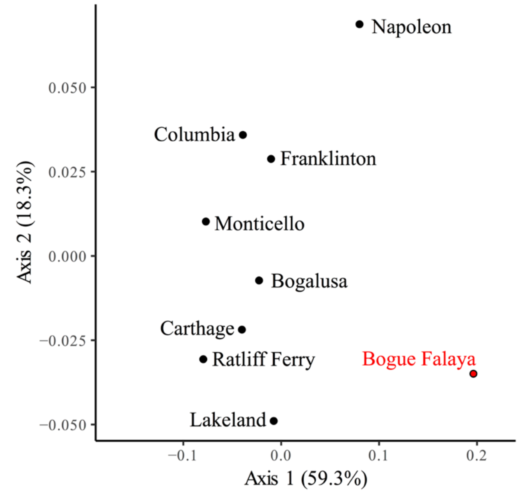

Figure 3. The first two axes from the Principal Coordinates Analysis of Nei's unbiased genetic distance (Nei 1978) of the microsatellite data for the nine Ringed Map Turtle (*Graptemys oculifera*) sites. Site names correspond to those from Gaillard et al. (2015) other than the addition of the Bogue Falaya from this study.

Falaya site also possessed the highest pairwise F_{ST} values (Appendix Table 1), which ranged from 0.091 (Napoleon-Bogue Falaya) to 0.170 (Ratliff Ferry-Bogue Falaya).

After editing, we retained 659 bp of sequence data for the mitochondrial control region, including two indels. Among the 12 individuals we sequenced (four from Bogue Falaya and eight from sites in the Pearl River), we found a total of five haplotypes. Two of the haplotypes matched those previously reported for *G. oculifera* (GenBank Accession numbers GQ25370-25371; Ennen et al. 2010) and three were new to this study (GenBank accession numbers OQ725693–OQ725695; Appendix Table 2). None of the new haplotypes were unique to the Bogue Falaya population, however. The first new haplotype (O1) was found in the individuals from Carthage, Franklinton, and Bogalusa and was only 1–2 substitutions different (uncorrected p distance 0.0015–0.0030) from the second new haplotype (O2) found in the individual from Ratliff Ferry. These two haplotypes were also only 1–2 substitutions (uncorrected p distance 0.0015–0.0030) different from haplotype GQ253570 (OCULIFERA_01). The remaining eight individuals (haplotype O3 - Bogue Falaya, Monticello, Napoleon, Columbia, and Lakeland) differed by only one base substitution from haplotype GQ253571 (OCULIFERA_02). The two parts of the haplotype network differed by 5–7 base substitutions (uncorrected p distance 0.0076–0.0107; Fig. 4).

Table 2. Average admixture scores (*q* scores) and associated standard error in parentheses from the STRUCTURE analyses of the Ringed Map Turtles (*Graptemys oculifera*) microsatellite data. Site names and numbers correspond with those from Gaillard et al. (2015) other than the addition of site #9 (Bogue Falaya) from this study.

Site Name	Avg *q* (SE) Group 1	Avg. *q* (SE) Group 2
Carthage (1)	0.98 (0.004)	0.02 (0.004)
Ratliff Ferry (2)	0.99 (0.001)	0.01 (0.001)
Lakeland (3)	0.90 (0.008)	0.10 (0.008)
Monticello (4)	0.93 (0.009)	0.07 (0.009)
Columbia (5)	0.95 (0.005)	0.05 (0.005)
Bogalusa (6)	0.92 (0.006)	0.08 (0.006)
Napoleon (7)	0.65 (0.059)	0.35 (0.059)
Franklinton (8)	0.97 (0.006)	0.03 (0.006)
Bogue Falaya (9)	0.01 (0.001)	0.99 (0.001)

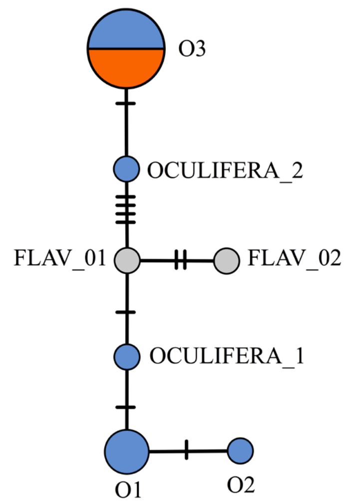

Figure 4. Minimum spanning network of the 659 bp mtDNA control region sequences of Ringed Map Turtle (*Graptemys oculifera*; OCULIFERA) and Yellow-blotched Map Turtle (*G. flavimaculata*; FLAV) from GenBank and the three new haplotypes (O1-O3) identified in this study. The size of the circle representing each haplotype reflects the combined frequency of the haplotype among our samples and the sequences from GenBank. Colors reflect the geographic location of the *G. oculifera* haplotypes, with blue representing sites in the Pearl River drainage and orange representing the Bogue Falaya site. Hash marks along branches indicate the number of mutational differences between adjacent haplotypes.

Discussion

Origins of the Bogue Falaya population.—Our results show that *G. oculifera* individuals from the Bogue Falaya constitute a natural and discrete population from all other *G. oculifera* in the Pearl River system. The Bogue Falaya is a major tributary of the Tchefuncte River and is physically isolated from the Pearl River system, as the two river mouths are about 65 km apart and characterized by unsuitable brackish marsh habitat in their lower reaches and brackish Lake Pontchartrain between them. Therefore, *G. oculifera* should no longer be considered endemic to the Pearl River system. The presence of *G. oculifera* in the Bogue Falaya likely reflects historical connectivity to the Pearl River either via lateral migration of the Pearl River over the course of the late Pleistocene (Heinrich 2006) or through a historical connection to the Pearl River during lower sea level periods (Swift et al. 1986). In either scenario, the Bogue Falaya population of *G. oculifera* seems to have been isolated long enough to become genetically distinct as measured by the microsatellite loci data. Especially given the lack of unique mitochondrial control haplotypes in the Bogue Falaya, however, we are not suggesting that this level of genetic differentiation in the microsatellite loci warrants taxonomic recognition. Similarly, Selman et al. (2013) found that the Escatawpa River population of *G. flavimaculata* was genetically distinct from the Pascagoula River, likely because of isolation of the river systems by sea level rise.

An unnoticed population.—How could a species go unnoticed by the scientific community until now in the middle of a city not far from New Orleans? There appear to be several reasons why this has occurred. First, following the study by Cagle (1953), there seems to have been significant inertia indicating that *G. oculifera* was endemic to the Pearl River. There is a fair amount of published literature on *G. oculifera* (e.g., Jones and Hartfield 1995; Jones 2006, 2017; Gaillard et al. 2015; Selman and Jones 2017). This is largely because of its federal listing in 1986 as Threatened under the ESA (USFWS 1986) and a subsequent recovery plan that outlined many needed studies (Stewart 1988). Because *G. oculifera* was believed to be endemic to the Pearl River system at the time of listing, that is where the subsequent studies occurred.

Second, the specimens now referred to as *G. oculifera* were described by Baur (1890) from a collection of turtles given to him by Gustave Kohn of New Orleans, Louisiana. The localities of the Louisiana turtles were reported as from Mandeville, Louisiana. Cagle (1953) stated that repeated attempts by field crews in 1947 and 1948 to collect *G. oculifera* near Mandeville and elsewhere in the

Florida Parishes of Louisiana failed, though it is not certain if they surveyed the relatively small section of the Bogue Falaya in Covington where we found *G. oculifera.* Cagle (1953) assumed that all of specimens of Kohn were taken from the Pearl River, as this is the only location where this species had been observed in previous studies. The Pearl River is about 42 km east of Mandeville, which is near the brackish Lake Pontchartrain, and Mandeville is about 12 km to the south-southeast of the Bogue Falaya in Covington where we collected *G. oculifera*. Smaller tributaries entering Lake Pontchartrain that are unlikely to contain *G. oculifera* exist near Mandeville (Bayou Chinchuba, Bayou Castine, and Bayou Cane), but perhaps the specimens of Kohn came from the Bogue Falaya. If this were the case, however, it is surprising that Mandeville was the stated origin and not Covington. Both dry and wet preparations of the original specimens are housed in the Smithsonian National Museum of Natural History. So perhaps with advances in molecular techniques it may be possible to discern the true origins of the specimens collected by Kohn. We do not know if *G. oculifera* collected by Kohn were from the Bogue Falaya population we now know to exist, or were they indeed mislabeled, and part of the Pearl River population as suggested by Cagle (1953).

***ESA considerations.*—**Often, federally listed species are rare and challenging to study due to low detection probabilities (Engler et al. 2004; Marini et al. 2010); however, this is not the case with *Graptemys*, which are gregarious while they bask (pers. obs.). Though *Graptemys* can be harder to capture in hand than many other turtles, they are easily detected through binoculars, spotting scopes, or a long zoom-lens cameras, and southern populations can be seen basking during every month of the year including warm days during the winter. The fact that this population of *G. oculifera* went unnoticed by the scientific community, especially in the middle of a city contained within the New Orleans-Metairie-Kenner metropolitan statistical area, underscores how basic surveys can help to fully understand distributional ranges. In fact, if it were not for the photographs taken by a citizen scientist and posted online, this *G. oculifera* population would still be hiding in plain sight. This study further highlights the role citizen science websites can serve in advancing scientific knowledge (Callaghan et al. 2022).

Being genetically distinct, the Bogue Falaya population of *G. oculifera* may warrant the designation of a distinct population segment (DPS), and under that designation, it would be managed separately from Pearl River system populations of *G. oculifera*. The three criteria used in an iterative process to designate a population as a DPS are discreteness, significance, and status (U.S. Fish and Wildlife Service and National Marine Fisheries Service 1996). The Bogue Falaya satisfies the first criterion of discreteness as this aquatic turtle has no current connection to populations in the Pearl River basin. It satisfies the significance criterion in that the loss of the Bogue Falaya population would result in a significant gap in the range of the species. In addition, further satisfying the significance criterion, we have shown that this population differs genetically from Pearl River populations. Lastly, because the Bogue Falaya population would likely be listed as Threatened or Endangered if it were its own species, it satisfies the status criterion to be designated a DPS. Regardless of any formal changes, the presence of an additional established population outside of the known Pearl River system provides redundancy that supports the ability of the species to withstand catastrophic events that may affect one system but not the other (Wolf et al. 2015).

Moving forward, with the identification of *G. oculifera* in a geographically separate river system, redundancy, resiliency, and representation can be re-assessed, and threats, like small population size and possible inbreeding depression, can be investigated in the Bogue Falaya population. A thorough population status assessment including the distribution, abundance, and threats to conservation of *G. oculifera* in the Bogue Falaya and the greater Tchefuncte River basin could help to inform management decisions. Future projects could also evaluate the potential hybridization risk with the congeneric Mississippi Map Turtle (*G. pseudogeographica kohnii*) that was also observed during tissue sampling efforts. The USFWS 5-year status review for *G. oculifera* is scheduled for fiscal year 2025 (Luke Pearson, pers. comm.). Gathering comprehensive information on this population can provide valuable data needed to assist with evaluating the current and future condition of the species and making informed decisions regarding conservation, management, and recovery for this threatened species.

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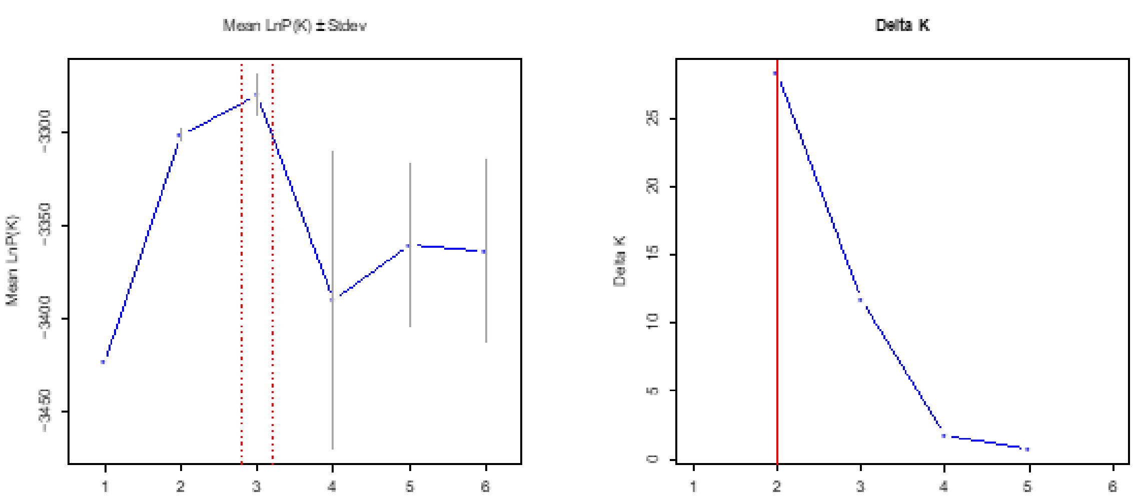
Appendices

Appendix Table 1. Pairwise F_{ST} values for the nine Ringed Map Turtle (*Graptemys oculifera*) sites. Site names correspond with those from Gaillard et al. (2015) other than the addition of site Bogue Falaya from this study.

	Carthage	Ratliff Ferry	Lakeland	Monticello	Columbia	Bogalusa	Napoleon	Franklinton	Bogue Falaya
Carthage	-								
Ratliff Ferry	-0.006	-							
Lakeland	0.003	0.007	-						
Monticello	0.026	0.022	0.025	-					
Columbia	0.018	0.026	0.022	0.005	-				
Bogalusa	-0.003	0.012	0.026	0.032	0.026	-			
Napoleon	0.076	0.099	0.076	0.066	0.039	0.060	-		
Franklinton	0.010	0.036	0.050	0.071	0.039	0.031	0.040	-	
Bogue Falaya	0.150	0.170	0.128	0.158	0.145	0.141	0.091	0.161	-

Appendix Table 2. Distances between Ringed Map Turtle (*Graptemys oculifera*) and Yellow-blotched Map Turtle (*G. flavimaculata*: FLAV) mitochondrial control region (659 bp) haplotypes. Uncorrected *p* distances are listed above the diagonal whereas the number of base substitutions is listed below the diagonal.

	FLAV_01	FLAV_02	OCULIFERA_01	O1	O2	OCULIFERA_02	O3
FLAV_01	-	0.0030	0.0015	0.0030	0.0046	0.0061	0.0076
FLAV_02	2	-	0.0046	0.0061	0.0076	0.0091	0.0107
OCULIFERA_01	1	3	-	0.0015	0.0030	0.0076	0.0091
O1	2	4	1	-	0.0015	0.0091	0.0076
O2	3	5	2	1	-	0.0107	0.0091
OCULIFERA_02	4	6	5	6	7	-	0.0015
O3	5	7	6	5	6	1	-



Appendix Figure. Results of the STRUCTURE analysis of Ringed Map Turtle (*Graptemys oculifera*) from Bogue Falaya and sites across the Pearl River system. The Log likelihood plot is presented first followed by the Delta K results.