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Cryptic diversity among Yazoo Darters (Percidae: *Etheostoma raneyi*) in disjunct watersheds of northern Mississippi

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The Yazoo Darter, *Etheostoma raneyi* (Percidae), is an imperiled freshwater fish species endemic to tributaries of the Yocona and Little Tallahatchie rivers of the upper Yazoo River basin, in northern Mississippi, USA. The two populations are allopatric, isolated by unsuitable habitat between the two river drainages. Relevant literature suggests that populations in the Yocona River represent an undescribed species but a lack of data prevents a thorough evaluation of possible diversity throughout the range of the species. Our goals were to estimate phylogenetic relationships of the Yazoo Darter across its entire distribution and identify cryptic diversity for conservation management purposes. Maximum likelihood and Bayesian phylogenetic analyses of the mitochondrial cytochrome *b* gene returned two reciprocally monophyletic clades consistent with individuals sampled from the two river drainages. Analyses of the nuclear *S7* gene yielded unresolved trees with mostly low support. No haplotypes were shared between the drainages for either gene. Additional cryptic diversity within the two drainages was not indicated. Estimated divergence between Yazoo Darters in the two drainages occurred during the Pleistocene (<1 million years ago) and was likely linked to repeated spatial shifts in suitable habitat and changes in watershed configurations during glacial cycles. Our results support a reevaluation of the taxonomic and conservation status of Yazoo Darters in the Yocona River drainage and provide essential data for proposed human-assisted gene flow among isolated populations within each river drainage.

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Abstract

The Yazoo Darter, *Etheostoma raneyi* (Percidae), is an imperiled freshwater fish species endemic to tributaries of the Yocona and Little Tallahatchie rivers of the upper Yazoo River basin, in northern Mississippi, USA. The two populations are allopatric, isolated by unsuitable habitat between the two river drainages. Relevant literature suggests that populations in the Yocona River represent an undescribed species but a lack of data prevents a thorough evaluation of possible diversity throughout the range of the species. Our goals were to estimate phylogenetic relationships of the Yazoo Darter across its entire distribution and identify cryptic diversity for conservation management purposes. Maximum likelihood and Bayesian phylogenetic analyses of the mitochondrial cytochrome *b* gene returned two reciprocally monophyletic clades consistent with individuals sampled from the two river drainages. Analyses of the nuclear *S7* gene yielded unresolved trees with mostly low support. No haplotypes were shared between the drainages for either gene. Additional cryptic diversity within the two drainages was not indicated. Estimated divergence between Yazoo Darters in the two drainages occurred during the Pleistocene (<1 million years ago) and was likely linked to repeated spatial shifts in suitable habitat and changes in watershed configurations during glacial cycles. Our results support a reevaluation of the taxonomic and conservation status of Yazoo Darters in the Yocona River drainage and provide essential data for proposed human-assisted gene flow among isolated populations within each river drainage.

Introduction

The southeastern United States has a globally significant amount of diversity among its freshwater fishes (Abell et al., 2008). A large portion of this diversity is contained within Etheostomatinae (Percidae), the darters (Jelks et al., 2008; Page & Burr, 2011). Though the group shows a wide variety of life history strategies and associated distributional patterns (Fluker, Kuhajda & Harris, 2014), many species of darters are range-limited (microendemics) (Page, 1983; Page & Burr, 2011) and share a suite of life history characteristics that are associated with limited dispersal (Turner & Trexler, 1998; Turner, 2001), including niche conservatism (Keck & Near, 2010). The discovery of microendemism in darters is occurring more frequently because, at least in part, the routine use of genetic tools is increasingly uncovering cryptic diversity (Hollingsworth & Near, 2009; April et al., 2011; Echelle et al., 2015; Kozal et al., 2017; Matthews & Turner, 2019).

The Yazoo Darter (*Etheostoma raneyi* Suttkus and Bart, 1994) is an Adonia clade snubnose darter (*sensu* Near et al., 2011) distributed in the upper Yazoo River basin in north-central Mississippi (Fig, 2, S1, S2, and S3). Surface geology mostly comprises highly erodible, unconsolidated sands and clays with resulting fine substrates within streams. Topography is relatively flat compared with upland regions but is more variable compared with the Lower Gulf Coastal Plain and Mississippi Alluvial Plain to the west (Ross, 2001; Keck & Etnier, 2005; Powers & Warren, 2009) (Fig. 1).Yazoo Darters occur in headwater tributaries of the Little Tallahatchie River and Yocona River whose confluence lies in bottomland habitat of the Mississippi Alluvial Plain, which is unfavorable for the darter. In common with other snubnose darters, Yazoo Darters are small (<65 mm Total Length), benthic insectivores lacking a swim bladder (Page, 1983; Johnston & Haag, 1996; Sterling, Warren & Henderson, 2013). Generaliz long distance movements for spawning or feeding are not documented for Adonia species. Larvae of Adonia darters, including the Yazoo Darter, are pelagic but active swimmers upon hatching and select for sheltered areas out of direct current immediately downstream of spawning areas; passive drift of larvae is not documented (Simon & Wallus, 2006; Ruble, Sterling & Warren, in press). A population genetic study of the Yazoo Darter using microsatellite data indicated limited historical dispersal among tributary streams and virtually no contemporary dispersal, likely because of anthropogenic habitat destruction. Genetic structure was high across small spatial scales among some tributary populations (F_{st} = 0.03-0.17) within each major drainage where the species occurs (Little Tallahatchie and Yocona rivers) and was also high between drainages (F_{st} = 0.17-0.29) (Sterling et al., 2012).

A phylogenetic analysis of Upper Gulf Coastal Plain Adonia darters in western Kentucky, Tennessee, and northern Mississippi indicated that Yazoo Darters inhabiting the Little Tallahatchie and Yocona rivers were genetically distinct and reciprocally monophyletic with high posterior support. However, the study was limited to six individuals from only a few streams in each drainage (Powers & Warren, 2009).

The Yazoo Darter is categorized as vulnerable by the American Fisheries Society (Jelks et al., 2008) and the Southeastern Fishes Council (Warren et al., 2000), as globally imperiled by the Nature Conservancy (NatureServe, 2019), as sensitive by the USDA Forest Service (USDA Forest Service, 2013), and as a Tier 1 species of greatest conservation need by the Mississippi State Wildlife Action Plan (Mississippi Museum of Natural Science, 2015). Human-assisted gene flow among tributaries within each drainage was recommended as a conservation management action (Sterling et al., 2012). However, more rigorous estimates of the distribution of genetically distinct populations are needed to inform such an action. We used two genetic markers, mitochondrial *cytb* and nuclear *S7* genes, to investigate possible cryptic diversity within each major drainage and to assess the results from Powers & Warren (2009) using larger sample sizes from sites across the distribution of the species.

Materials and Methods

We sampled 117 individuals from 20 streams representative of the entire range of the Yazoo Darter via single-pass backpack electrofishing, dip nets, and seines (Fig. 2). ~~Collecting~~ localities included nine streams in the Yocona River drainage and 11 streams in the Little Tallahatchie River drainage. For spatial comparisons within drainages, we categorized sample sites in the Yocona River drainage as either within the Yocona River watershed (YR hereafter) or the Otoucalofa Creek watershed (a large tributary of the Yocona River; Otoucalofa hereafter). In the Little Tallahatchie River drainage we categorized sites within the Cypress Creek watershed (Cypress hereafter), Tippah River watershed (Tippah hereafter), and Little Tallahatchie River watershed (LTR hereafter) (Fig. 2; Tables 1, S1, and S2). We obtained tissue samples by either taking pelvic fin clips or by collecting voucher specimens, which we stored in 95% ethanol at -74^{o}C. This study was conducted with the approval of the University of Mississippi IACUC Committee (protocol 09-027), using annual collection permits from the Mississippi Museum of Natural Science (2009-2017: 0604091, 0513101, 0624112, 0622122, 0602132, 0610142, 0624151, 0715163, 1010173).

We isolated whole genomic DNA (MacManes, 2013) and used previously published PCR primers to amplify the entire mitochondrial *cytb* gene (1140 bp; Song et al., 1998) and the forward sequence of intron 1 of the nuclear *S7* ribosomal gene (599 bp; Chow & Hazama, 1998). PCR components were as follows: 9.8µl ddH_2O, 0.2µl dNTP, 0.4µl $MgCl_2$, 2µl 5x reaction buffer, 0.2µl each 10 nM primer, 0.15µl Phire™ *Taq*, and 1.5 µl of template DNA (~15µl total reaction volume). We set conditions for PCR reactions as 98° C for 30 s, followed by 30 cycles of 98° C for 6 s, 53.1-56° C for 30 s, and 72° C for 60 s. We purified and sequenced PCR products using ExoSAP-IT (ThermoFisher Scientific) and Big Dye (ver. 3.1, ThermoFisher Scientific) according to manufacturer's recommendations. Arizona State University DNA sequencing facility processed the samples (https://asu.corefacilities.org/service_center/show_external/3900/asu-dna-lab) using an automated ABI 3730 sequencer. We assembled all resulting forward and reverse sequences into contigs for each individual and aligned them using MEGA (ver. 7.0.26; Kumar, Stecher & Tamura, 2016). We obtained outgroup sequence data and sequences for two additional Yazoo Darters (Near et al., 2011), one from each major drainage, from GenBank for use in our analyses. Sequence data for this study are available from GenBank (https://www.ncbi.nlm.nih.gov/nuccore/, Tables S1, S2, and S3).

We used PartitionFinder V 1.1.1 (Lanfear et al., 2012) to find the best-fit model for each locus. The *cytb* dataset was partitioned by 1st, 2nd, and 3rd codon positions, and the *S7* dataset was analyzed as a single partition. We analyzed partitioned datasets for each gene (Tables S1, and S2) using Bayesian Inference (BI) implemented in MrBayes ver. 3.2.6 (Ronquist et al., 2012) via CIPRES Science Gateway ver. 3.3 (https://www.phylo.org/) (Miller et al., 2010). Each partition/analysis included the most appropriate substitution models for the two loci as suggested by PartitionFinder. We used two runs of MrBayes for 10^6 generations; four Markov chains sampled every 10,000 steps and Tracer (ver. 1.7.1; Rambaut et al., 2018) removed 25% of the posterior trees as burn-in. We then generated a consensus tree in MrBayes. We used the same data to construct Maximum Likelihood (ML) trees using RAxML-HPC ver. 8.0 (https://cme.h-its.org/exelixis/web/software/raxml/) (Stamatakis, 2014) also using the CIPRES Science Gateway ver. 3.3 (Miller et al., 2010). We used the default GTR model and performed 100 bootstrap replicates to assess nodal support. We considered nodes with posterior probabilities ≥95% as strongly supported (Huelsenbeck & Ronquist, 2001).

We visualized relationships among individuals using haplotype networks (TCS v. 1.21; Clement et al., 2000) for each gene. We estimated uncorrected pairwise genetic distances (p-distances) ~~generated~~ in MEGA ver. 7.0.26 (Kumar, Stecher & Tamura, 2016) between drainages and among watersheds within drainages for each gene. For comparison, we also generated p-distances among all Adonia clade snubnose darters using our data and publicly available *cytb* gene~~tic~~ sequences (see Table S4 for genetic sequence data).

We calculated the number of haplotypes, and haplotype diversity for both loci using DNAsp V 5.10 (Librado & Rozas, 2009) between drainages and among watersheds within drainages. We derived estimates of divergence times from rates of molecular evolution of the *cytb* and *S7* genes reported by Near et al. (2011), and p-distance values given by MEGA for *S7* and *cytb*.

Results

The most appropriate substitution models for the 1st, 2nd, and 3rd codon positions of the *cytb* (1139 nucleotides (nt) in length) were F81, GTR+G, and K80+I and for the *S7* gene (530 nt), F81+G. Results from Bayesian and ML analyses for *cytb* indicate two monophyletic clades congruent with the two river drainages (Fig. 3). Support for reciprocally monophyletic clades was high for the ML analysis (bootstrap support: Yocona River, 95%; Little Tallahatchie River, 100%), but only moderately supported for the Bayesian analysis (posterior probabilities: Yocona River, 0.12; Little Tallahatchie River, 1.0). Phylogenetic trees generated using the *S7* gene generally lacked support for samples from the Little Tallahatchie River drainage and were not consistent between the Bayesian and ML analyses, though samples from the Yocona River drainage were ~~returned as~~ a single clade with high support (95% bootstrap support and 0.97 posterior probability). Haplotype networks for *S7* and *cytb* indicate that no haplotypes were shared between drainages (Fig. 4). Fifteen genetic characters are diagnostic of Yazoo Darters in the two major river drainages (Table 2).

Uncorrected p-distance (*cyt b*) between drainages was 0.8% and among watersheds within drainages was 0.01% in the Yocona River drainage and 0.1 and 0.11% in the Little Tallahatchie River drainage (Table 3). For comparison, p-distances (*cytb*) among Adonia snubnose darters ranged from 0.5-14.53% (Tables 4-S5). P-distances (*S7*) between drainages was 0.3% and among watersheds within drainages was 0.01% in the Yocona River drainage and ranged from 0.07-0.17% in the Little Tallahatchie River drainage (Table 3).

Haplotype diversity was higher in the Little Tallahatchie River drainage (*cyt b*: Hd = 0.66, 11 haplotypes; *S7*: Hd = 0.48, 2 haplotypes) than in the Yocona River drainage (*cyt b*: Hd = 0.11, 3 haplotypes; *S7*: Hd = 0.06, 2 haplotypes). Estimated times of divergence were 0.88 (*S7*) and 0.44 my (*cyt b*).

Discussion

Our results indicate two major findings, 1) there is no evidence of species-level diversity within each major river drainage; 2) however, there is support for recognizing Yazoo Darter populations in the Yocona River drainage as a distinct species under the phylogenetic species concept (Nixon & Wheeler, 1990).

The overall mismatch between genetic clades and geography within drainages could be explained by a lack of lineage sorting over time, chance events such as extreme flooding moving genes among tributaries, or possibly, changes in stream configurations. Because of the low support at most within-drainage nodes (phylogenetic trees), the only inference that can be made is that the genetic markers used did not reveal genetically distinct within-drainage populations. In contrast, microsatellite markers indicated that tributaries do contain genetically distinct populations with a strong isolation by distance effect (Sterling et al., 2012). Taken together, patterns of gene flow within drainages apparently have not been stable over enough generations to produce a clear signal in the *cytb* and *S7* data.

Some measure of pairwise genetic sequence divergence is usually reported in phylogenetic studies, but differences in methods can make direct comparisons problematic, which was the motivation for our estimation of genetic distances among all forms of Adonia darters. Though some distance values between closely related species were similar to the values we report for Yazoo Darters, others were surprisingly high and more similar to values among forms that are putatively more distantly related (Table 4, Near et al., 2011). Our results indicate that distance measures are useful only insofar as they are placed into a larger, comparable context. Even so, the values we report are similar to measures reported for other sister fish species (Johns & Avise, 1998).

Our results show that genetic divergence (haplotype diversity, Hd) is much lower in the Yocona River drainage than in the Little Tallahatchie River drainage. This is consistent with previous genetic studies (Powers & Warren, 2009; Sterling et al., 2012) and has obvious conservation management implications.

The lack of resolution for the *S7* analyses are not surprising given that population divergence likely occurred <1 my. In young clades of darters, *cytb* usually has more power to resolve relationships with higher support relative to other genetic markers with slower mutation rates and higher effective population sizes (Avise, 2004; Keck & Near, 2008) and our results are consistent with other studies (Keck & Near, 2010; Echelle et al., 2015; Kozal et al., 2017). This is apparently true for closely related Adonia clade darters in western Tennessee and Kentucky (Kozal et al., 2017), which is interesting because the vicariant events that led to isolation of ancestral populations and ensuing divergence among Upper Gulf Coastal Plain populations currently recognized as *E. cervus*, *E. pyrrhogaster*, and *E. zonistium* almost certainly were a factor in divergence of ancestral Yazoo Darters in the upper Yazoo River basin (Powers & Warren, 2009) (Fig. 1). This is supported by divergence time estimates for those three species that are similar to our estimates (0.4-1.4 my, Kozal et al., 2017).

We propose that spatial shifts in suitable habitat for Yazoo Darters during repeated glacial cycles over the last 2 my led to the isolation of populations in the upper Yocona and Little Tallahatchie River drainages (see Hewitt, 1996). During glacial periods and low sea levels, suitable habitat for Yazoo Darters would have expanded downstream, perhaps into the present Mississippi Alluvial Plain when sea levels were at their lowest (90-140 m below present). Streams were smaller (less precipitation), entrenched in bedrock, and had higher gradients, coarse substrate, and cold, clear water. During interglacial periods sea levels rose, streams had more water, gradients moderated, stream valleys filled in with fines, and streams were no longer confined to bedrock. Suitable habitat for Yazoo Darters would have moved upstream. As suitable habitat shifted up- and downstream in the Yazoo River Basin, connectivity among groups of Yazoo Darters in tributary streams would also have changed. During interglacial periods when streams were not confined to bedrock, changes in stream configurations seem more likely, especially in headwaters. However, during glacial periods, streams were smaller (climate was much drier) and confluences lower in the watershed were less likely to be barriers to dispersal because they were smaller and were suitable habitat for Yazoo Darters. Dispersal among tributaries under these conditions seems more likely (Fisk, 1944; Rittenour, Blum & Goble, 2007; Past Interglacials Working Group of PAGES, 2016).

Spatial changes in the downstream extent of suitable habitat likely interacted with changes in the location of the ancient confluence of the Yocona and Little Tallahatchie Rivers to isolate Yazoo Darter populations. Reliable data exists for estimating the number, duration, and timing of glacial and interglacial periods over about the last 800,000 years (Past Interglacials Working Group of PAGES, 2016). An estimated 11 cycles between glacial and interglacial periods are identified. Estimated duration of interglacial periods is much shorter (166,700 years) than glacial and transitional periods (633,300 years) (Fisk, 1944; Past Interglacials Working Group of PAGES, 2016). Given this setting, downstream connectivity among demes would have likely had greater influence structuring Yazoo Darter populations between the two major drainages than possible shifts in stream configurations. Further, changes to the position of the confluence of the Little Tallahatchie and Yocona rivers and with the ancient predecessors of the Ohio and Yazoo rivers were likely instrumental in the phylogenetic pattern seen in our results (Fisk, 1944). It seems apparent that at some point (about 0.4-0.8 my) suitable habitat for Yazoo Darters no longer encompassed the confluence of the Yocona and Little Tallahatchie Rivers during glacial periods.

Our results help to refine the management actions (i.e., human-assisted gene flow) suggested in Sterling et al. (2012). Though we did not find genetically distinct groups that were consistent with geography within the Little Tallahatchie drainage, our results clearly indicate that there are no deeply divergent populations within the two major drainages. Based on our results and those in Sterling et al. (2012), we recommend ~~relocation~~ of individuals among tributaries that are closest together within watersheds (as categorized in this study). Within the Yocona River watershed we recommend restricting movement of individuals to within the Otoucalofa Creek watershed or adjacent tributaries to the mainstem Yocona River. Since genetic diversity was higher in the Little Tallahatchie River than in the Yocona River, consistent with previous results (Powers & Warren, 2009; Sterling et al., 2012), and because populations in the Yocona River face greater risks (see Introduction), the relocation of individuals within the Yocona River should be considered. Research aimed at identifying mechanisms of gene flow are also desperately needed for the Yazoo Darter, which would also help inform management of other imperiled forms of Adonia snubnose darters.

Our results indicate that populations of the Yazoo Darter in the Yocona River drainage are an undescribed, genetically distinct species. Though there are no obvious differences in pigment patterns or color between the populations in each drainage, Suttkus et al. (1994) noted modal differences in lateral line scale counts and Sterling et al. (2013) showed that Yazoo Darters in the Yocona River drainage are significantly longer than those in the Little Tallahatchie River drainage. Further investigation of morphology, meristics, and pigment patterns is warranted.

Acknowledgments

We would like to thank M. Bland, Z. Barnett, A. Carson, C. Smith, B. Sterling, W. Sterling, and G. McWhirter for help collecting samples. We are also grateful to J. Hubbell and J. Schaefer for sharing data and G. Henderson for help with figures. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

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Figure 1

~~Map showing the~~ distribution of Adonia clade snubnose darters in the Upper Gulf Coastal Plain of Kentucky, Tennessee, and Mississippi (southeastern United States).

Major river systems and physiographic provinces discussed in the text are also pictured (see map legend).

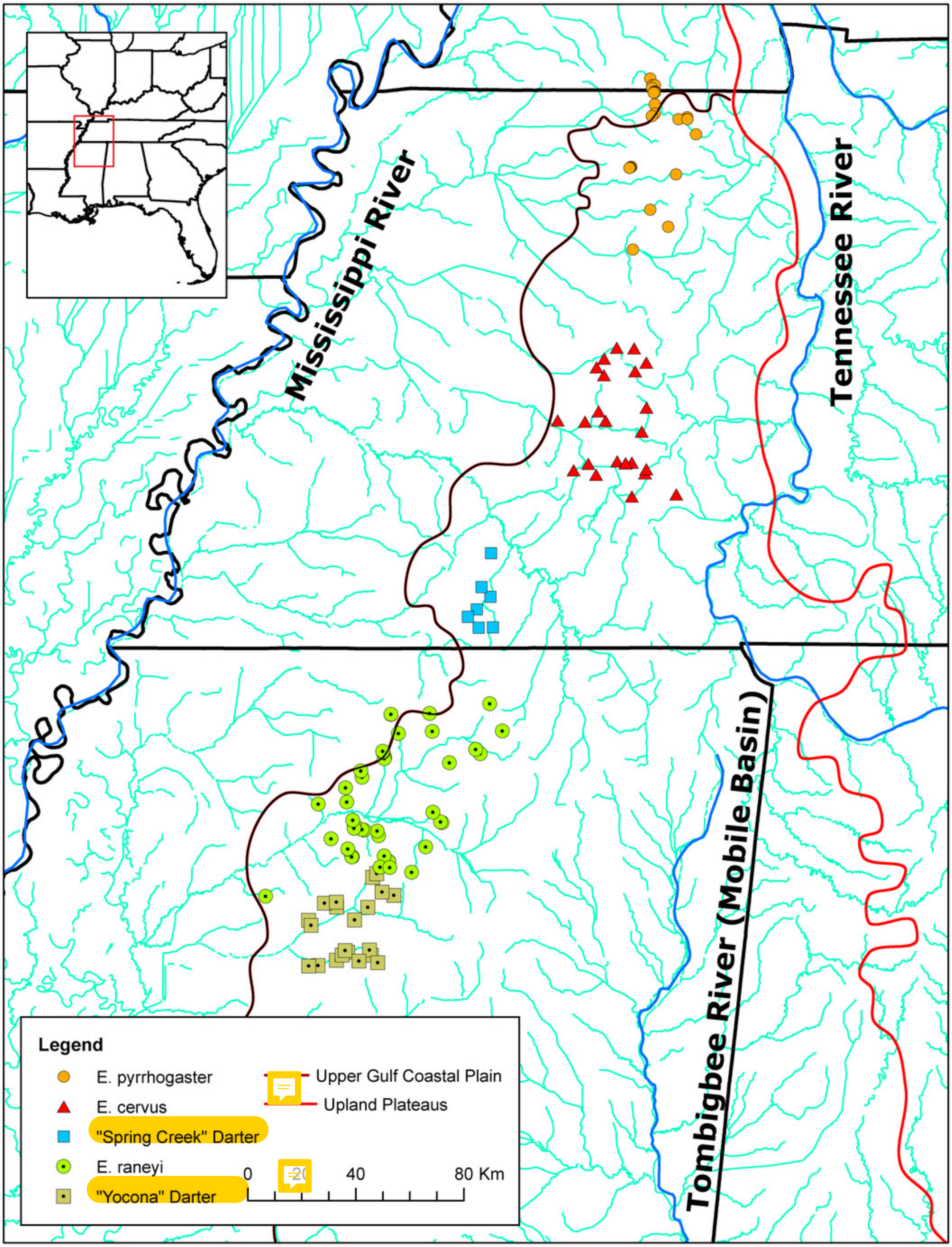
Mississippi River
Tennessee River
Tombigbee River (Mobile Basin)
Legend
E. pyrrhogaster
E. cervus
"Spring Creek" Darter
E. raneyi
"Yocona" Darter
Upper Gulf Coastal Plain
Upland Plateaus
0
20
40
80 Km

Figure 2

Map of the upper Yazoo River basin showing genetic tissue sample sites in the Little Tallahatchie River (Yazoo Darter) and Yocona River (“Yocona Darter”) drainages.

Numbers correspond to data in Table 1.

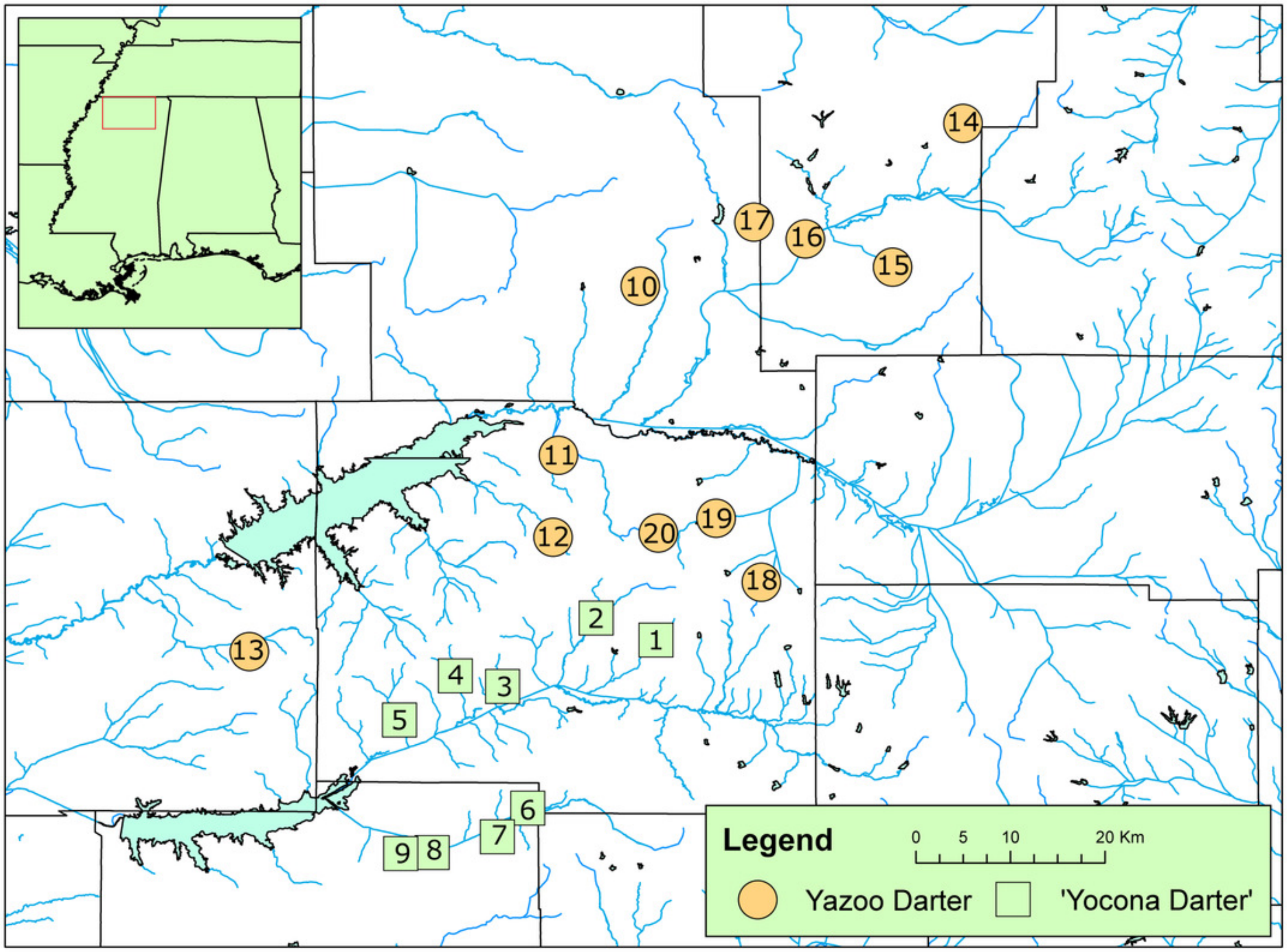

Figure 3

Phylogenetic tree of the partitioned *cytb* dataset using Bayesian estimation (MrBayes ver. 3.2.6).

Bayesian posterior probabilities are above nodes and maximum likelihood bootstrap values (RAxML-HPC ver. 8.0) are below nodes (see Tables S1 and S2 for sequence data).

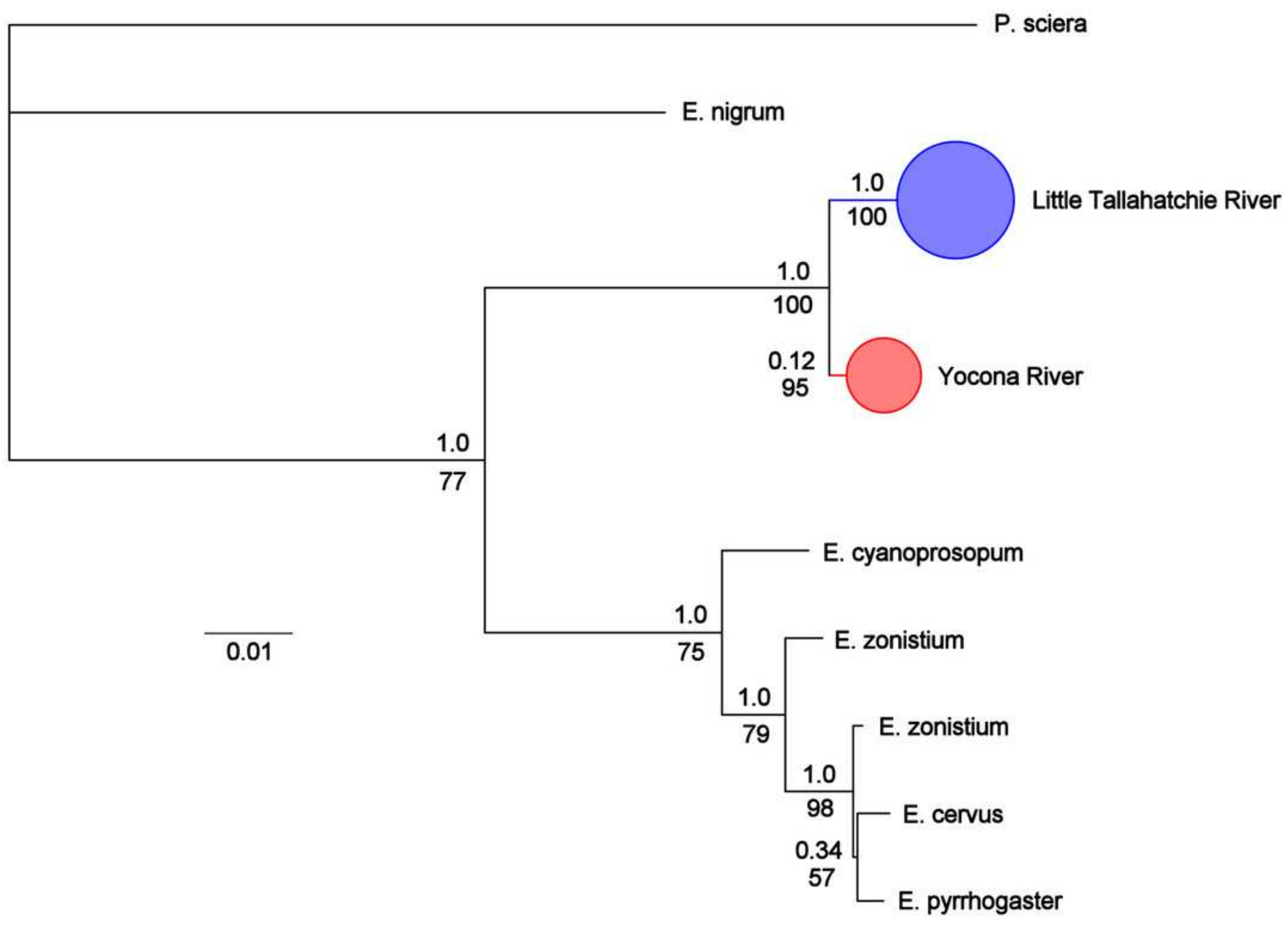

Figure 4

S7 (A) and *cytb* (B) gene trees for samples among the two major drainages and watersheds within drainages.

Red and blue indicates the Yocona and Little Tallahatchie river drainages, respectively.

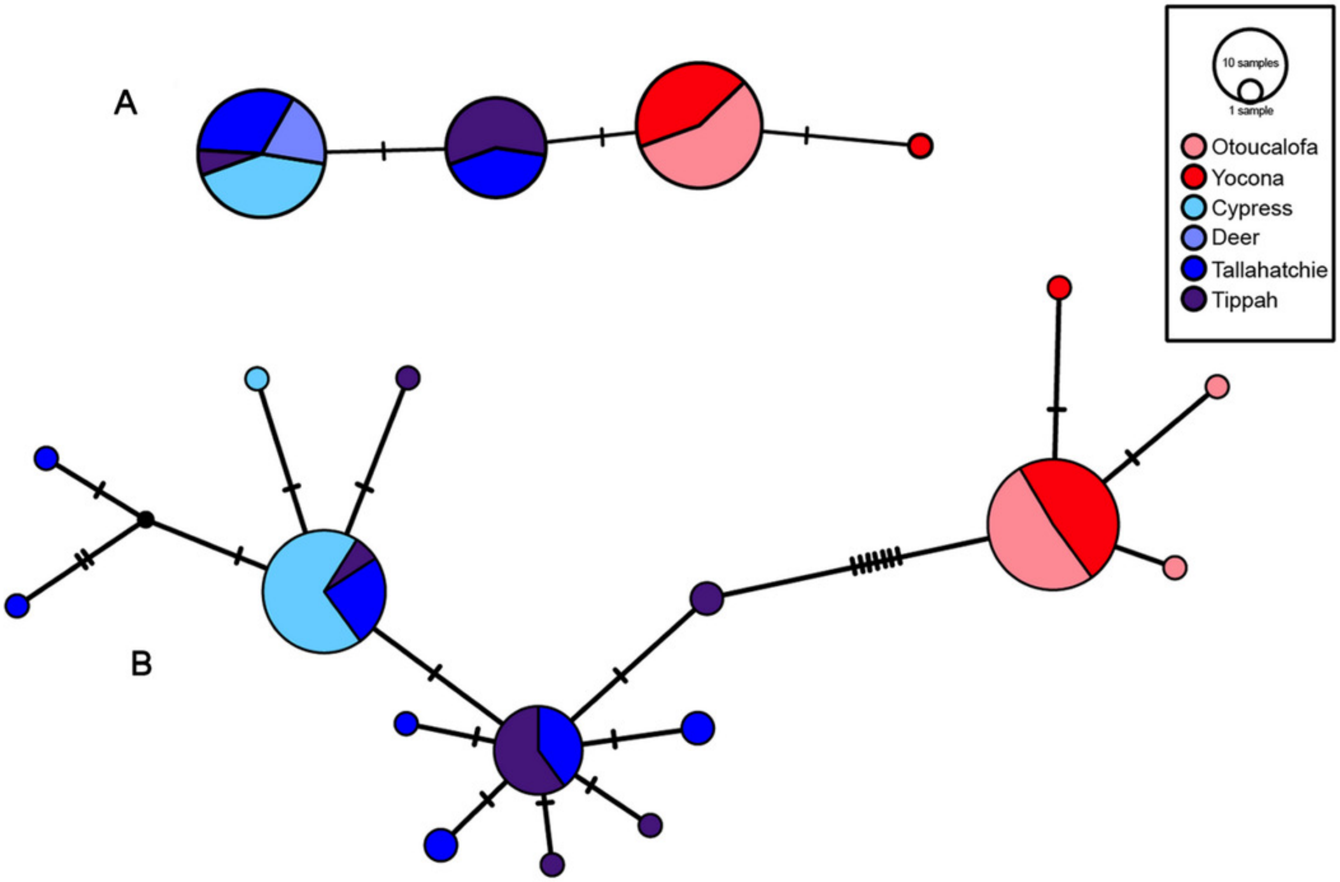

Table 1(on next page)

Genetic tissue sample data for each of the two major drainages within the distribution of the Yazoo Darter.

Watershed groups discussed in the text are given, as well as sample locations and sample sizes for genetic analyses. Site ID numbers correspond to Fig. 2; see Tables S1 and S2; U.T. = unnamed tributary.

Site ID	Drainage	Watershed	Stream	*Cytb*, n	*S7*, n	Latitude	Longitude
1	Yocona River	YR	Pumpkin Creek	4	1	34.327	-89.398
2	Yocona River	YR	Yellow Leaf Creek	2	1	34.348	-89.455
3	Yocona River	YR	Morris Creek	4	4	34.283	-89.544
4	Yocona River	YR	Taylor Creek	5	5	34.293	-89.589
5	Yocona River	YR	Splinter Creek	3	4	34.251	-89.642
6	Yocona River	Otoucalofa Creek	Mill Creek	6	6	34.167	-89.52
7	Yocona River	Otoucalofa Creek	Gordon Branch	3	2	34.14	-89.549
8	Yocona River	Otoucalofa Creek	U.T. Otoucalofa Creek	4	3	34.125	-89611
9	Yocona River	Otoucalofa Creek	Johnston Creek	6	6	34.124	-89.641
10	Little Tallahatchie River	LTR	Big Spring Creek	10	13	34.664	-89.413
11	Little Tallahatchie River	LTR	Graham Mill Creek	3	3	34.503	-89.491
12	Little Tallahatchie River	LTR	Hurricane Creek	2	3	34.425	-89.496
13	Little Tallahatchie River	LTR	Deer Creek	6	6	34.316	-89.785
14	Little Tallahatchie River	Tippah River	Yellow Rabbit Creek	5	4	34.819	-89.106
15	Little Tallahatchie River	Tippah River	Chilli Creek	5	4	34.682	-89.173
16	Little Tallahatchie River	Tippah River	U.T. Tippah River	2	2	34.709	-89.256
17	Little Tallahatchie River	Tippah River	Chewalla Creek	4	3	34.725	-89.305
18	Little Tallahatchie River	Cypress Creek	Cypress Creek	7	6	34.382	-89.298
19	Little Tallahatchie River	Cypress Creek	Puskus Creek	12	6	34.443	-89.341
20	Little Tallahatchie River	Cypress Creek	Bay Springs Branch	2	1	34.429	-89.396

Table 2(on next page)

Genetic characters that diagnose allopatric populations of Yazoo Darters in the Little Tallahatchie River and Yocona River using mitochondrial cytochrome b (*cytb*) and nuclear *S7* genes.

Numbers indicate the location of the character along the genetic sequence; A = adenine, C = cytosine, T = thymine, G = guanine.

Character (*cyt b*)	**L. Tallahatchie**	**Yocona**
147	A	G
165	C	T
585	C	T
588	T	C
654	A	G
876	G	A
897	A	G
930	G	A
1056	A	G
1090	G	A
1107	G	A
1113	G	A
Character (S7)	**L. Tallahatchie**	**Yocona**
286	G	A
insertion: 478	G	-
insertion: 479	C	-

Table 3(on next page)

Uncorrected pairwise distances (p-distance, %) among watersheds.

Values for *cytb* are given below the diagonal and for *S7* above.

	Otoucalofa Creek	YR	LTR	Tippah River	Cypress Creek
Otoucalofa Creek		0.01	0.35	0.24	0.41
YR	0.01		0.36	0.25	0.43
LTR	0.82	0.81		0.13	0.07
Tippah River	0.76	0.75	0.11		0.17
Cypress Creek	0.83	0.82	0.10	0.10	

Table 4(on next page)

Uncorrected p-distances (%), among closely related Adonia clade snubnose darters.

Species complexes are grouped following Near et al. (2011). Bold type and asterisk = values <2.0%; Fk. = Fork, Cr. = creek; see Table S3 for complete data.

	E. raneyi (Yazoo Darter group)		E. zonistium (Bandfin Darter group)				
	"Yocona"	E. raneyi	E.zonistium	"Spring Cr."	E. cervus	E. pyrrhogaster	E. cyanoprosopum
"Yocona"							
E. raneyi	**0.75***						
E.zonistium	7.61	8.07					
"Spring Creek"	8.33	8.64	**1.29***				
E. cervus	8.45	8.97	**1.42***	**0.50***			
E. pyrrhogaster	8.61	9.04	**1.44***	**0.72***	**0.86***		
E. cyanoprosopum	8.84	9.24	4.25	4.75	4.86	4.84	
E. bellator	8.50	8.99	8.93	9.38	9.48	9.39	9.84
E. chermocki	8.22	8.71	8.74	9.19	9.29	9.20	9.62
"Locust Fork"	9.70	10.18	9.64	9.81	9.80	9.81	10.24
"Sipsey"	10.29	10.40	10.69	11.02	10.99	10.84	11.02
"Conasauga"	8.79	8.71	9.03	9.32	9.41	9.46	9.51
"Amicalola"	7.84	7.95	7.78	8.08	8.17	8.41	8.20
E. brevirostrum	8.79	8.71	9.22	9.51	9.60	9.68	9.78
E. simoterum	14.33	14.24	15.03	15.01	15.04	15.42	15.01
Percina sciera	16.91	16.81	16.81	17.30	17.46	17.47	17.11

	E. bellator (Warrior Darter group)				E. brevirostrum (Holiday Darter group)		
	E. bellator	E. chermocki	"Locust Fk."	"Sipsey"	"Conasauga"	"Amicalola"	E. brevirostrum
E. bellator							
E. chermocki	**0.57***						
"Locust Fork"	5.11	4.92					
"Sipsey"	6.24	6.05	6.57				
"Conasauga"	8.79	8.60	9.22	9.84			
"Amicalola"	7.45	7.07	7.69	8.21	3.15		
E. brevirostrum	8.88	8.69	9.89	10.12	**1.05***	3.63	
E. simoterum	14.33	14.14	13.90	14.52	14.90	13.94	14.71
Percina sciera	15.85	15.57	15.62	17.03	17.77	17.00	17.86