5-2012

Interspecific Hybridization Among Black Basses (Micropterus spp.) in the Chattahoochee River, Columbus Georgia

Kimberly S. Holley
Columbus State University, holley_kimberly@columbusstate.edu

INTERSPECIFIC HYBRIDIZATION AMONG BLACK BASSES (MICROPTERUS SPP.) IN THE CHATTAHOOCHEE RIVER, COLUMBUS, GEORGIA

Kimberly Sheena Holley

Columbus State University

The College of Letters and Sciences

The Graduate Program in Environmental Science

Interspecific hybridization among black basses (*Micropterus* spp.) in the Chattahoochee River, Columbus, Georgia

A Thesis in

Environmental Science

By

Kimberly Sheena Holley

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Master of Science

May 2012

I have submitted this thesis in partial fulfillment of the requirements for the degree of Master of Science.

10 May 2012
Date

Sheena Holley
Kimberly Sheena Holley

We approve the thesis of Kimberly Sheena Holley as presented here.

10 May, 2012
Date

K S. B
Kevin S. Burgess, Assistant Professor of Biology, Thesis Advisor

10 May '12
Date

W L Birkhead
William Birkhead, Professor Emeritus of Biology, Thesis Advisor

10 May 2012
Date

G R Moyer
Gregory R. Moyer, Regional Geneticist
U.S. Fish and Wildlife Service
Warm Springs Fish Technology Center

ABSTRACT

Interspecific hybridization can lead to the evolution of a new hybrid species or extinction of parental species due to competition or excessive backcrossing. When parental populations differ in abundance, hybrids tend to backcross more frequently with the more abundant parent, resulting in asymmetrical introgression. The objective of this study was to determine the extent and apparent direction of hybridization between shoal bass (*Micropterus cataractae*), a rare endemic species to the Apalachicola drainage, and spotted bass (*M. punctulatus*), an introduced and more abundant species. Pelvic fin tissue (N = 130) was taken from bass species in the Chattahoochee River between Columbus, Georgia and Phenix City, Alabama and analyzed for hybridization using a combination of four microsatellite markers, morphometrics, and mtDNA. While morphometrics proved to be inadequate at confirming hybridization, microsatellite analyses identified 15.4% (N = 20) of bass samples as hybrids. Analysis also showed significantly more hybrids backcrossed to the more abundant *M. punctulatus*, suggesting asymmetrical introgression. Mitochondrial DNA were utilized to confirm asymmetrical introgression; instead, barcoding revealed the potential for three additional interspecific interactions involving *M. floridanus* (Florida bass), *M. coosae* (redeye bass), and *M. salmoides* (largemouth bass). Although mtDNA analysis did not confirm hybridization between *M. cataractae* and *M. punctulatus*, interspecific hybridization does pose a threat to populations of *M. cataractae* and warrants additional research. To protect populations of *M. cataractae*, priorities should focus on restoring shoal habitat and if necessary augmenting existing populations with genetically, pure shoal bass from within their range.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEGEMENTS

I would first like to thank Dr. William Birkhead for encouraging me to pursue research and helping me develop my thesis topic. I would also like to thank you for your guidance and expertise in field work. Without your help, this project would have never landed at my feet.

I would like to thank Dr. Kevin Burgess for taking me in as a graduate student, without any prior interest in fish work. Despite your love for plants, you were able to cross over to animals and be an amazing resource. Thank you for fostering this research idea and finding a means for me to conduct lab work. I would also like to thank you for the extensive hours you have given to me in helping with data analysis and interpretation.

I would also like to thank Dr. Gregory Moyer and Ashantye' Williams for their generosity in opening their genetics lab to me. Thank you so much for teaching me everything I needed to know from DNA extractions to data analysis for genotypes. You have provided me with an immense amount of knowledge and I am very grateful.

To Brian Hickson and Devin Chappell, thank you for offering your time, expertise, and electrofishing boat. Because of you, I was able to collect specimens for data analysis that the CSU electrofishing boat could have never shocked. Your eagerness to drive 40 miles with a boat and stay all day in the Chattahoochee River made this project possible.

To my fellow graduate students, Andy Hartzog and Danny Walker, I would like to thank you for your support throughout my research as well as your involvement in fish collections.

I would like to thank Dr. William Frazier, Crystal Jones, and the Environmental Science Department of Columbus State University for providing me the funds to travel to Warm Springs, GA to work in their genetics lab. Also, I would like to thank you for the funds granted to present my research at the Southeastern Fishes Council and Association of Southeastern Biologists meetings.

Lastly, I would like to thank my family and friends for supporting me through my graduate studies. Thank you so much for keeping me motivated and well fed in order to complete this thesis.

INTRODUCTION

Interspecific hybridization can have multiple consequences, which can be disproportionally detrimental when one parental species is rare while the other is more abundant (Arnold 1997, Burgess *et al.* 2005). Hybridization can lead to the evolution of a new hybrid species through speciation or extinction of parental species due to competition and/or excessive backcrossing (Leary *et al.* 1995, Rhymer & Simberloff 1996, Pierce & Van Den Avyle 1997, Martinsen *et al.* 2001). Speciation occurs when there are low barriers to reproduction, allowing new species to evolve (Hubbs 1955, Allendorf & Luikart 2007). When a parental species is rare or has a low population size, hybrids can potentially outcompete the rare parental species, causing extripation or extinction of the parental species (Rhymer & Simberloff 1996, Burke & Arnold 2001). Parental extinction can also result when hybrids backcross to either parent or other hybrids. When parental populations differ in abundance, hybrids tend to backcross more frequently with the more abundant parent, resulting in asymmetrical introgression, or unidirectional gene flow (Arnold 1992, Morizot *et al.* 1991, Anderson 1948 & 1949, Rawson & Hilbish 1998, Wolf *et al.* 2001, Burgess *et al.* 2005). Through repeated rounds of backcrossing, genetic assimilation can occur, whereby there is a loss of the rare parental taxon (Rhymer & Simberloff 1996, Arnold 1997). In addition, hybrids acquire a higher genetic contribution from the more abundant parent. In either case, hybridization can be a threat to the genetic integrity of parental taxon (Leary *et al.* 1995, Albert *et al.* 1997, Pierce & Van Den Avyle 1997).

In North American fishes, hybridization has been well documented, primarily due to introductions of nonindigenous species (Rhymer & Simberloff 1996, Perry *et al.*

2002). Some of the most aggressively introduced species are black bass of the genus *Micropterus* for sports fishing (Morizot *et al.* 1991, Pierce & Van Den Avyle 1997, Pipas & Bulow 1998, Goclowski 2010). Historically, interspecific hybridization among black basses was rare in nature (Hubbs & Bailey 1940, Whitmore 1983, Pipas & Bulow 1998, Morizot *et al.* 1991). However, fish stocking has allowed formerly allopatric species to encounter one another, which has readily led to hybridization and genetic introgression within the genus (Morizot *et al.* 1991, Koppelman 1994, Pierce & Van Den Avyle 1997, Pipas & Bulow 1998, Kassler *et al.* 2002, Barwick *et al.* 2006, Littrell *et al.* 2007).

The shoal bass, *Micropterus cataractae*, is a fluvial specialist endemic to the Apalachicola drainage in Alabama, Georgia, and Florida (Williams & Burgess 1999, Sammons & Maceina 2009, Goclowski 2010), with introductions in 1975 into the upper Ocmulgee River in Georgia (Bart *et al.* 1994, Wheeler & Allen 2003). Historically, their range included most of the Chattahoochee and Flint River basins. However, *M. cataractae* is currently listed as a species of special interest over their entire range by the American Fisheries Society (Williams *et al.* 1989), threatened in Florida (Gilbert 1992), and most recently listed as a species of high conservation concern in Alabama (Mirarchi *et al.* 2004). Populations are declining due to habitat loss from impoundments, siltation, pollution, poor land use, altered stream hydrology (Williams & Burgess 1999), possible competition with similar bass species (Maceina *et al.* 2007, Sammons & Maceina 2009), and potential hybridization with spotted bass (*M. punctulatus*; Dakin *et al.* 2007, Maceina *et al.* 2007, Tringali *et al.* 2010, Goclowski 2010).

Many areas historically inhabited by *M. cataractae* are now dominated by the generalist *M. punctulatus* (Sammons & Maceina 2009). *M. punctulatus* was first

introduced in the Apalachicola drainage prior to 1941 by anglers and continue to be illegally introduced into systems across the range of *M. cataractae* (Williams & Burgess 1999, Sammons & Maceina 2009). Competition between *M. cataractae* and *M. punctulatus* is not considered a major threat to populations of *M. cataractae* (Goclowski 2010); however, the possibility of introgressive hybridization between the two species does exist and has sparked this current research (Dakin *et al.* 2007, Maceina *et al.* 2007, Tringali *et al.* 2010, Goclowski 2010). Potential hybridization has been cited due to similar habitat preferences and overlapping spawning times within the same stream systems (Ramsey & Smitherman 1972, Goclowski 2010, Birdsong *et al.* 2010).

To date, no genetic work has been conducted on the potential hybridization of *M. cataractae* and *M. punctulatus* within the mainstream of the Chattahoochee River in Georgia. Within Alabama, two putative *M. cataractae* and *M. punctulatus* hybrids were genetically confirmed within Osanippa Creek, a tributary to the Chattahoochee River (D. Philipp, Illinois Natural History Survey, unpublished data). Extensive research has been conducted in the Chipola River, where genetic markers were developed and used to determine whether hybridization was occurring between these two species. Tringali *et al.* (2010) found 5 hybrids (2% hybridization rate) between *M. cataractae* and *M. punctulatus*, all of which were backcrosses to *M. cataractae*. Unfortunately, the direction of introgression was never assessed.

The purpose of the present study is to access the extent and apparent direction of hybridization within and between populations of *Micropterus cataractae* and *M. punctulatus*. By using a combination of morphometric, microsatellite, and mitochondrial DNA analyses, the following questions will be addressed: (i) What is the frequency of

hybrids formed between shoal and spotted bass in nature? (ii) Is there evidence of asymmetrical hybridization? (iii) Does morphological variation have a genetic basis?

MATERIALS AND METHODS

Study site and sampling

Sampling was conducted during fall 2010, between 5 October and 27 October, and spring 2011, between 11 April and 6 May, along three sites on the Chattahoochee River between Columbus, GA and Phenix City, AL (N 32.4694° W -84.9971°). The first site was a free-flowing stream sector between Dillingham Bridge and Eagle and Phenix Dam. The latter two sites were impounded sectors between the Eagle and Phenix and City Mills Dams and between City Mills and North Highlands Dams, respectively.

Putative *M. cataractae* (n = 25), *M. punctulatus* (n = 249), and hybrid bass (n = 11) were randomly sampled using a boat-mounted electrofisher or a Smith-Root 12-B backpack electrofisher, depending on the depth and ease of access of the river. Fishes were classified initially using classical morphological descriptions: relative depth of dorsal notch, extension of mouth terminus (upper jaw bone), presence of a tooth patch on tongue, presence of dark spots below the lateral line, caudal fin color, and eye color (Mettee *et al.* 1996, Boschung & Mayden 2004). Pelvic fin tissue was collected from each individual using Warm Springs, GA FWS Conservation Genetics Lab Standard Operating Procedures (2011) and stored on ice while in the field. Upon returning to the lab, samples were stored in a -20°C freezer until further lab work was conducted.

DNA isolation and amplification

Four microsatellite markers were used in this study: Msa-06, Msa-10, Msa-22, and Msa-32 (Tringali *et al.* 2010; Table 1). These markers were chosen because they have nearly species-specific alleles that allow identification between *M. cataractae* and *M. punctulatus* (Tringali *et al.* 2010). Genomic DNA from 130 samples [*M. cataractae* (n = 24), *M. punctulatus* (n = 95 randomly selected), hybrid bass (n = 11)] was extracted from pelvic fin tissue frozen at -20°C and preserved in ethanol using a QIAGEN DNeasy Tissue Kit for 96 well plates. Whole genomic DNA was quantified using a nano-spectrophotometer (Thermo Scientific). Samples exceeding 100 ng/µl were diluted using molecular grade water and requantified.

DNA was amplified in 8 µl reaction mixtures containing 0.672 µl $MgCl_2$ (2 mM), 1.26 µl dNTPs (1.5 mM), 0.42 µl of forward and reverse primers (0.5 µM), 1.05 µl buffer (1 mM), 0.09 µl *Taq* DNA polymerase (0.0855 unit/µl) (Applied Biosystems, Inc.), 3.44 µl distilled water, and 1 µl genomic DNA (20-100 ng). Using a GeneAmp PCR system 9700 (Applied Biosystems, Inc.), PCR amplifications were performed under the following conditions: initial denaturing at 94°C for 10 min, followed by a touchdown method involving 33 cycles of denaturing at 94°C, and annealing and extension at 74°C, where the initial annealing temperature was 56°C and decreased 0.2°C per cycle.

Microsatellite analysis

PCR products were co-loaded by mixing 2 µl of 2:100 dilution of PCR product with an 8 µl solution consisting of 97.5% HIDI formamide and 2.5% Genescan-500 LIZ size standard (Applied Biosystems, Inc.), followed by denaturing for 3 min, and standard refrigeration for 5 min. Microsatellite alleles were visualized using an ABI 3130 genetic

analyzer (Applied Biosystems, Inc.) with fluorescently labeled forward primers and scored using GeneMapper Software version 3.7 (Applied Biosystems, Inc.).

To detect hybridization, STRUCTURE version 2.3.3, a Bayesian clustering method, was used to analyze all 130 individuals (Pritchard *et al.* 2000). Parameters were set to default using an admixture model with no prior population information, correlated allele frequencies, and 95% probability intervals. The number of potential genetic clusters (K) was set to 2 to account for the two parental species (Zalapa *et al.* 2010). Length of burn-in was set to 20,000 steps followed by 10^5 iterations.

Individuals were assigned to one of two possible genetic clusters according to their admixture coefficient (q), as follows: $q > 0.983$ represented individuals belonging to parental species (shoal or spotted bass) (Tringali *et al.* 2010); $0.41 < q < 0.59$ indicated F_1 hybrids (Albarrán-Lara *et al.* 2010); and $0.983 < q < 0.6$ were backcrosses. Because the $q > 0.983$ cutoff was based off the Tringali *et al.* (2010) data set that incorportated additional loci, backcrosses may go undetected in this study. To explore hybrid assignment, individuals were reassigned using $q > 0.95$ and $q > 0.99$ as cutoffs to hybridity and compared to the prior cutoff model.

A Chi-Square goodness of fit test was used to determine if the proportion of spotted alleles present in hybrids, using $q > 0.983$ as the cutoff to hybridity, differed significantly from a 1:1 ratio.

Morphometric analysis

A Chi-Square test of independence was used to compare the distribution of population clusters as determined by microsatellite and morphometric analyses.

Morphotypes were compared used structural and color traits: relative depth of dorsal notch, extension of mouth terminus, presence of a tooth patch on tongue, presence of dark spots below the lateral line, caudal fin color, and eye color (Appendix A). Traits were analyzed using a Chi-Square test of independence between and among the three putative groups as determined by microsatellite analysis.

Mitochondrial DNA sequence

To determine the maternal inheritance of hybrids, the 5' region of the cytochrome c oxidase 1 (COI) gene was sequenced in a subsample of putative *M. cataractae* (n = 9), *M. punctulatus* (n = 18), and hybrid bass (n = 13) that were identified from the STRUCTURE analysis ($q > 0.983$ cutoff). Approximately 652 bp were amplified from the COI gene using the following primer set (Ward *et al.* 2005):

FishF2-5'TCGACTAATCATAAAGATATCGGCAC

FishR2-5'ACTTCAGGGTGACCGAAGAATCAGAA

With modifications to Ivanova *et al.* (2007) protocol, amplifications were performed in 12.5 µl reaction mixtures containing 6.25 µl trehalose (5 µM), 1.25 µl buffer (1 µM), 1.25 µl $MgCl_2$ (2.5 mM), 0.625 µl of forward and reverse primers (1 µM), 0.25 µl dNTPs (0.2mM), 0.25 µl Ampli*Taq* Gold DNA polymerase (1.25 unit/µl) (Applied Biosystems, Inc.), and 2 µl genomic DNA (20-100 ng). PCR amplifications were conducted using a Mastercycler® pro thermocyler (Eppendorf vapo.protect) under the following conditions: 94°C for 2 min followed by 35 cycles of 94°C for 30 s, 52°C for 40 s, and 72°C for 1 min, with an extension at 72°C for 10 min (Ward *et al.* 2005, Ivanova *et al.* 2007). Bidirectional sequencing was conducted at Functional Biosciences, Inc. (Madison, WI) on an ABI 3730xl DNA Sequencer (Applied Biosystems, Inc.) using

the BigDye Terminator version 3.1 cycle Sequencing Kit (Applied Biosystems, Inc.). Sequences were edited and assembled using CodonCode Aligner version 3.7.1 (Codoncode Corporation) then imported into Geneious Pro version 4.8.5 (Drummond *et al.* 2011) for alignment. Edited sequences were submitted to Genbank (Benson *et al.* 2011) and the Barcode of Life Database (BOLD) (Ratnasingham & Hebert 2007) to confirm identities, and a neighbor-joining tree was constructed.

RESULTS

Microsatellite analysis

A total of 130 individuals were sampled, with 18.5% (n = 24) classified as *M. cataractae*, 66% (n = 86) as *M. punctulatus*, and 15.4% (n = 20) as hybrids, according to the $q > 0.983$ cutoff to hybridity. In comparison, the $q > 0.95$ cutoff to hybridity identified 22% (n = 29) as *M. cataractae*, 71% (n = 92) as *M. punctulatus*, and 7% (n = 9) as hybrids. The $q > 0.99$ cutoff identified 13% (n = 17) as *M. cataractae*, 49% (n = 64) as *M. punctulatus*, and 38% (n = 49) as hybrids (Fig. 1). The $q > 0.983$ cutoff, as used in the Chipola River study (Tringali *et al.* 2010), was chosen as a modest predictor of hybridity; therefore, further analyses were conducted using only this cutoff.

Of the 20 hybrids, 30% (n = 6) backcrossed to *M. cataractae* and 70% (n = 14) backcrossed to *M. punctulatus*, with no F1 hybrids detected. Hybridization was bidirectional; however, there were significantly more backcrosses to *M. punctulatus*, suggesting asymmetrical introgression ($X^2 = 68.14$, d.f. = 8, $P < 0.001$; Fig. 2).

Microsatellite results showed no species specific alleles; however, there were six alleles with allele frequencies ≥ 0.304 distinguishing *M. cataractae* from *M. punctulatus*

(Table 3, Appendix B). Three of which were highly specific to *M. cataractae* and the remaining three identified *M. punctulatus.*

Morphometric analysis

The distribution of genetic clusters produced with microsatellites was not significantly different than the distribution determined by morphological identification (X^2 = 3.02, d.f. = 2, P > 0.05; Fig. 3). However, identifying *Micropterus* to species level using morphology was successful only 67.7% of the time.

M. cataractae and *M. punctulatus*, identified by microsatellite analyses, were significantly different in regards to morphological characters, except for caudal fin and eye color. Dorsal notch and mouth morphologies differed significantly between *M. cataractae* and *M. punctulatus*, with hybrids being intermediate (X^2 = 15.54, d.f = 2, P <0.0005; X^2 = 13.56, d.f = 2, P <0.005; Fig. 4a-b; Table 4). Presence of a tooth patch and black lateral spots were significantly higher in *M. punctulatus* than in *M. cataractae* and hybrids, which did not differ (X^2 = 25.04, d.f = 2, P <0.0001; X^2 = 26.03, d.f = 2, P <0.0001; Fig. 4c-d; Table 4). There was no significant difference in the distribution of caudal fin and eye colors present among the three species clusters (Fig. 5a-b).

mtDNA analysis

Of the thirty five (87.5%) COI sequences (604 bp) obtained from *Micropterus* samples, 27 (77%) were bidirectional contigs while 8 (23%) had unidirectional reads (Appendix C). Based on BLASTn analyses to Genbank and BOLD, COI results deviated from the microsatellite results (Table 5). Of the 9 samples identified by microsatellite as *M. cataractae*, 2 were identified as *M. cataractae*, 3 as *M. salmoides* (largemouth bass), and

the remaining 4 as *M. floridanus* (Florida bass). All 15 samples identified by microsatellite as *M. punctulatus* were identified as *M. coosae* (redeye bass). Of the 11 samples identified by microsatellite as hybrids, 5 (45.5%) contained the *M. punctulatus* COI sequence and 1 (9%) contained the *M. cataractae* COI sequence. The remaining 5 putative hybrids consisted of 2 (18.2%) *M. salmoides* and 3 (27.3%) *M. coosae* sequences.

Sample identities were based off of BLASTn percent identity scores ≥ 99.5%. Within each species identified by mtDNA sequencing, percent pairwise identities were also ≥ 99.5%, with fewer than 3 basepair differences except for *M. cataractae* (5 bp differences) and *M. coosae* (6 bp differences) (Fig. 6).

A Neighbor-joining tree of mtDNA sequences showed *M. coosae* and *M. cataractae* as most similar to *M. salmoides* and *M. floridanus*, with *M. punctulatus* being the most genetically distinct species (Fig. 7-8).

DISCUSSION

In eight of the nine species of *Micropterus*, hybridization has been documented as a result of species introductions outside their native ranges (Whitmore 1983, Kassler *et al.* 2002, Tringali *et al.* 2010). Hybridization and introgression can severely impact the genetic integrity of species in the genus, specifically those species considered rare due to narrow native ranges (Littrell *et al.* 2007). Among those species at risk includes *Micropterus cataractae*, a rare endemic to the ACF watershed, whose native range is now dominated by the introduced *M. punctulatus*. The primary goal of this study was to determine if hybridization has occurred between *M. cataractae* and *M. punctulatus* in the mainstream

of the Chattahoochee River and if so, does the genetic composition of those hybrids favor the more abundant bass, *M. punctulatus*.

Hybridity analysis

Microsatellite analyses indicate that hybridization between *M. cataractae* and *M. punctulatus* has occurred, with twenty individuals identified as hybrids. These results show a much higher population of hybrids than a similar study in the Chipola River that found only a five hybrids between *M. cataractae* and *M. punctulatus* (Tringali *et al.* 2010). The discrepancy between these studies could be a result of differences in the number of markers analyzed. Tringali *et al.* (2010) utilized 14 markers while this study used only four of the markers they developed. While pure populations require fewer markers, Tringali *et al.* (2010) concluded that additional markers maybe necessary to determine introgressed individuals. While past studies have been successful at classifying hybrids using fewer than six loci (Moizot *et al.* 1991, Koppelman 1994, Pierce & Van Den Avyle 1997), the success is determined by the extent of frequency differences at alleles for each locus (Allendorf & Luikart 2007). Therefore, microsatellite results within the present study should be viewed with caution until further loci can be analyzed.

Within this study, backcrossing was bidirectional, with significantly more hybrids backcrossed to the more abundant parental, *M. punctulatus*. These results support asymmetrical introgression towards the more abundant parental due to unequal parental abundances. Conversely, Tringali *et al.* (2010) found all hybrids backcrossed to the rarer parental, *M. cataractae*. In both studies, introgression had occurred; however, differences in the direction of backcrossing may be the result of river locality and ecology. Populations of *M. cataractae* within the Chipola River may not be as severely imperiled

as in the Chattahoochee River; therefore, allowing for greater backcrossing to *M. cataractae*.

Neither this study nor that of Tringali *et al.* (2010) found F1 hybrids, which could reflect infrequent interactions between the two parental species or high selection against the F1 generation (Koppelman 1994). Rarity of F1 hybrids could suggest hybridization may be the result of uncommon breakdowns of reproduction barriers, such as unseasonably high flow pushing taxa into each others spawning grounds (Koppelman 1994). In addition to a lack of F1 hybrids, this study only found three adult hybrids, which may support a high selection against or genetic instability of hybrids. However, the disportionate number of juveniles could also be the result of the sampling time of year. Since the direction of backcrossing differed between the studies with no F1 hybrids detected, there can be no conclusions on superior fitness relative to *M. cataractae* and *M. punctulatus*.

Due primarily to the use of only categorical traits, morphometric analyses proved to be inadequate at identifying hybrids between *M. cataractae* and *M. punctulatus*. In fact, no characteristic was specific to either parental species. However, with the exception of caudal fin and eye color, *M. cataractae* and *M. punctulatus* had significantly different morphological characteristics in terms of the proportions observed. Due to the potential of asymmetrical backcrossing to *M. punctulatus*, hybrids would be expected to resemble *M. punctulatus*, which did not occur. Hybrids favored *M. cataractae* in regards to a moderate presence of a tooth patch and black lateral spots within the populations. The proportion of hybrids with either a shallow dorsal notch or a modest extension of the mouth terminus was intermediate between that of *M. cataractae* and *M. punctulatus*.

Although morphology has been used in the past as a classical identifier of hybridity, morphological analyses are limited (Koppelman 1994, Rhymer & Simberloff 1996, Pierce & Can Den Avyle 1997, Pipas & Bulow 1998), and even multivariate analyses have failed at distinguishing backcrosses from parental species (Whitmore 1983). Therefore, additional genetic analyses are recommended to confirm hybridization and the direction of introgression in *Micropterus* species.

mtDNA discrepancies

In this study, mtDNA barcoding was utilized to corroborate asymmetrical introgression as suggested by microsatellite analyses. Hybrids backcrossed to either parental species were expected to carry the genome of the respected parental. However, mtDNA results determined that instead of a simple *M. cataractae* and *M. punctulatus* hybridizing complex, three additional species, *M. floridanus*, *M. coosae*, and *M. salmoides,* may be involved. It is important to note that reference barcodes could be wrongly identified on both GenBank and BOLD, causing the misidentification of samples in this analysis.

Since barcoding analyses showed the potential for more than two distinct genetic clusters, additional STRUCTURE analyses were conducted using all microsatellite data with K values from 1 to 10. The most probable K value can be determined by comparing the posterior probability calculated for each K value. According to Pritchard & Wen (2004), the most probable K value is the value at which the posterior probabilities start to plateau. This analysis favored K = 5, supporting the barcoding analysis. However, species still did not cluster as predicted by GenBank and BOLD suggesting that there is a greater extent of hybridization occurring with other *Micropterus* species than previously

expected and highlighting the need to increase the number of microsatellite loci and to include other *Micropterus* species in any subsequent analyses.

Another important finding of the mtDNA analysis was the presence of *M. floridanus* (Florida bass) mtDNA and confirmation of *M. punctulatus* within the Chattahoochee River in Columbus, GA. The presence of *M. floridanus* mtDNA is indicative of the intergrade zone found between *M. salmoides* and *M floridanus* throughout Georgia, and eastern Alabama (Boschung and Mayden 2004); subsequent microsatellite analyses could be used to confirm the presence of pure or hybrid *M. floridanus* individuals. Barcoding also confirmed the presence of *M. punctulatus* within the Chattahoochee River in Columbus, GA. Recently, there has been much dispute over whether *M. henshalli*, Alabama bass, a former subspecies of *M. punctulatus*, have invaded the mainstream of Chattahoochee River south of the Fall Line (Bud Freeman, pers. comm. Nov. 10, 2011, Birdsong *et al.* 2010). *M. henshalli* were first recorded in the Chattahoochee River in the 1970s and have become established in major tributaries of the Chattahoochee River as well as in the mainstream of the Chipola River (Birdsong *et al.* 2010). Unfortunatly, Genbank and BOLD both lack COI reference sequences for *M. henshalli*. However, after bringing specimens back to the lab for additional morphometric analyses, traditional morphology suggested specimens to be *M. punctulatus* (Bud Freeman, pers. comm. May 4, 2012). Recent analyses have found *M. cataractae* to be more closely related to *M. henshalli* than *M. punctulatus* (Kassler *et al.* 2002). This suggests the hybridization between *M. cataractae* and *M. henshalli* may pose a greater risk to *M. cataractae* than hybridization between *M. cataractae* and *M. punctulatus* (Kassler *et al.* 2002, Birdsong *et al.* 2010).

Phylogenetic analyses have also determined that *M. henshalli* and *M. coosae* are a sister taxa and have been reported to hybridize within the Keowee Reservoir, SC (Kassler *et al.* 2002, Barwick *et al.* 2006). These results are significant to this study because all parental *M. punctulatus* barcoded were identified as *M. coosae*, possibly due to misidentification because both exhibit a patch of teeth on the tongue (Williams & Burgess 1999). Fortunately, this study suggests that *M. henshalli* have not established themselves in the Chattahoochee River in Columbus, GA; therefore, hybridization between *M. henshalli* and *M. coosae* does not pose a great threat to native *M. coosae* populations within this study area. However, there have been concerns that *M. cataractae* and *M. coosae* are capable of hybridizing; although, no studies have confirmed hybridization (Dunham *et al.* 1994, Bart *et al.* 1994). Instead, the two species occur sympatrically within the Chattahoochee River catchment through resource partitioning (Williams & Burgess 1999, Birdsong *et al.* 2010).

In addition to *M. coosae*, *M. salmoides* are also endemic throughout the range of *M. cataractae* and were collected in this study (Williams & Burgess 1999). *M. cataractae* and *M. salmoides* are naturally sympatric and exhibit habitat partitioning to reduce competition. As confirmed in this study, *M. cataractae* inhabit fast-moving, shoal habitat with rocky substrate while *M. salmoides* prefer slow-moving pools with sandy bottoms (Wheeler & Allen 2003). Despite habitat barriers, *M. cataractae* and *M. salmoides* have been found to hybridize within the Chipola River (Tringali *et al.* 2010). Six *M. cataractae* and *M. salmoides* hybrids were detected, one of which was a F1 hybrid and the remaining five were backcrossed to *M. cataractae*. Anecdotal evidence also suggests that *M. salmoides* and *M. punctulatus* have hybridized at low frequencies in Halawakee

Creek, Alabama (D. Philipp, Illinois Natural History Survey, unpublished data). Within this study of the Chattahoochee River, sixty putative *M. salmoides* were analyzed in addition to *M. cataractae* and *M. punctulatus* using microsatellite loci (Msa-06, Msa-10, Msa-22, and Msa-32). However, STRUCTURE analyses, using K values from 1 to 10, were unable to cluster known *M. salmoides*, as determined by mtDNA analysis. According to posterior probabilities, results obtained here indicate that five genetic clusters are appropriate for this study. As mentioned earlier, discrepancies in clustering could be due to extensive hybridization among multiple *Micropterus* species.

In order to better understand the genetic relationships within *Micropterus*, additional sampling and genetic analyses are required. Specifically, collecting tissue samples from pure populations of each species involved are needed to create parental references. Currently, barcode references for *M. henshalli* are lacking, which may have resulted in the misidentification of individuals in this study. Barcoding individuals initially would also serve to identify pure parentals. To assess hybridization, increasing the number of microsatellite loci analyzed is required due to additional parental species present in the study site.

Implications

Despite being listed as a species of special interest throughout their range, *M. cataractae* are managed differently within each state. Alabama populations are held to the strictest regulations with a complete moratorium on harvest of *M. cataractae* since October 2006 (Maceina *et al.* 2007). However, it is nearly impossible to manage or enforce regulations because of the difficulty in differentiating between species of the genus as well as their hybrids (Birdsong *et al.* 2010). In addition to evaluating the potential hybridization

between *M. cataractae* and *M. punctulatus*, a separate goal of the research was to develop field techniques for identifying *Micropterus* to species level. Specifically, efforts were made to distinguish *M. cataractae* from other species in the genus in hopes that fishers could adopt these standard identifiers. Within the current study, the use of classical morphological descriptions to identify specimens was successful only 67.7% of the time. Unfortunately, morphological characteristics collected for analyses were not species specific and were inadequate at identifying hybrids between *M. cataractae* and *M. punctulatus*. Therefore, further research is needed to provide accurate and quick identifiers for each species within *Micropterus*.

Stocking for sports fishing has played a critical role in aiding introductions of formerly allopatric congeners, thereby enhancing interspecific hybridization. In order to provide responsible management practices, the potential for introgression must be evaluated prior to stocking fish. Greater concern should be taken to determine which species are distinct and what are the consequences of introgression. In the past, *M. salmoides* and *M. floridanus* were stocked interchangeably as a single species, which has lead to introgression and a loss of genetic integrity within *M. salmoides* (Kassler *et al.* 2002). To prevent the same fate for *M. cataractae*, research is needed on the ecology and extent of hybridization between species throughout its entire range. Because hybridization is a form of competition between species, it may result in introduced species, such as *M. punctulatus*, replacing native *M. cataractae* (Koppleman 1994).

Priorities should focus on the conservation of genetically pure *M. cataractae*, through a combination of restoring habitat and stocking pure shoal bass. Although hybridization between *M. cataractae* and *M. punctulatus* has been documented, declines

in *M. cataractae* populations are primarily due to fragmentation and loss of habitat, through impoundments (Williams & Burgess 1999, Dakin *et al.* 2007, Tringali *et al.* 2010). Management of isolated populations is critical for the long term persistence of the species. Isolated populations face loss of genetic diversity and also increased risks of hybridization (Dakin *et al.* 2007, Birdsong *et al.* 2010). To deter hybridization, efforts are needed to maintain habitat diversity and protect rare shoals to allow for habitat partitioning within *Micropterus* (Wheeler & Allen 2003). Fortunately, a river habitat restoration project within this study area is in progress. Two, formerly hydroelectric dams, Eagle and Phenix Dam and City Mills Dam, will be removed, creating shoal habitat and providing fish-passage between two previous, separated populations of *M. cataractae* (Eubanks & Buckalew 2005). Through the use of stocking, *M. cataractae* populations have been positively affected within the Chattahoochee River below Morgan Falls in Atlanta, GA (T. Ingrams, GDNR, unpublished data) as well as in the Flint River south of Lake Blackshear in Warwick, GA (Long & Martin 2008). Therefore, after the removal of two Columbus, GA dams, stocking within these areas is recommended to augment existing *M. cataractae* populations. However, management should be cautious in stocking only genetically confirmed, pure *M. cataractae* with known holotypes.

LIST OF REFERENCES

Albarrán-Lara AL, *et al.* (2010) Leaf fluctuating asymmetry increases with hybridization and introgression between *Quercus magnoliifolia* and *Quercus resinosa* (Fagaceae) through an altitudinal gradient in Mexico. *International Journal of Plant Sciences*, **171**, 310-322.

Albert ME, D'Antonio CM, Schiernbeck KA (1997) Hybridization and introgression in *Carpobrotus* spp. (*Aizoaceae*) in California I. Morphological Evidence. *American Journal of Botany*, **84**, 896-904.

Allendorf FW, Luikart G (2007) *Conservation and the genetics of populations*. Blackwell Publishing, Malden, Massachusetts.

Anderson E (1948) Hybridization of the habitat. *Evolution*, **2**, 1-9.

Anderson E (1949) *Introgressive Hybridization*. Wiley, New York.

Arnold ML (1992) Natural hybridization as an evolutionary process. *Annual Review of Ecology and Systematics*, **23**, 237-261.

Arnold ML (1997) *Natural Hybridization and Evolution*. Oxford University Press, New York.

Bart HL Jr. *et al.* (1994) New distribution records of gulf slope drainage fishes in the Ocmulgee River system, Georgia. *Southeastern Fishes Council Proceedings Dedicated to the Preservation of Southeastern Fishes*, **1994**, 4-10.

Barwick DH, Oswald KJ, Quattro JM, Barwick RD (2006) Redeye bass (*Micropterus Coosae*) and Alabama spotted bass (*M. punctulatus henshalli*) hybridization in Keowee Reservoir. *Southeastern Naturalist*, **5**(4), 661-668.

Benson DA, Karsh-Mizrachi I, Lipman DJ, Sayer EW (2011) GenBank. Nuclear Acids Research, **39** (Database issue), D32–D37.

Birdsong T *et al.* (2010) A business plan for the conservation of native black bass species in the southeastern US: A ten year plan. Submitted as a Keystone Initiative to the National Fish and Wildlife Foundation.

Boschung HT, Mayden RL (2004) *Fishes of Alabama.* Smithsonian Books, Washington DC.

Burgess KS, Morgan M, Deverno L, Husband C (2005) Asymmetrical introgession between two *Morus* species (*M. alba*, *M. rubra*) that differ in abundance. *Molecular Ecology*, **14**, 3471-3483.

Burke JM, Arnold ML (2001) Genetics and fitness of hybrids. *Annual Review of Genetics*, **35**, 31-52.

Dakin EE, Porter BA, Freeman BJ, Long JM (2007) Genetic integrity of an isolated population of shoal bass (*Micropterus cataractae*) in the upper Chattahoochee River basin. Natural Resources Technical Report NPS/NRWRD/NRTR—2007/366. National Park Service, Water Resources Division Fort Collins, Colorado.

Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A (2011) Geneious v4.8.5, Available from http://www.geneious.com/.

Dunham RA *et al.* (1994) Isozyme variation of redeye and shoal bass populations in Georgia. Southeastern Cooperative Regional Project on Genetics and Breeding of Fishes under Georgia Federal Aid in Sport Fish Restoration Act. Dingell-Johnson Project F-42. Georgia.

Eubanks MJ, Buckalew JO (2005) Chattahoochee River Restoration: Removal of City Mills and Eagle and Phenix Dams. *Preceedings of the 2005 Georgia Water Resources Conference*. ed. K.J. Hatcher.

Gilbert CR (ed) 1992 Shoal bass, *Micropterus* n. sp. of *coosae*. Pp. 98-104 *in* Rare and Endangered Biota of Florida. Volume II: Fishes ed. C.R. Gilbert. Gainesville: University Press of Florida.

Goclowski MR (2010) Relations between shoal bass and sympatric congeneric basses in the Flint River, Georgia. MA thesis. Auburn University, Alabama.

Hubbs CL, Bailey RM (1940) A revision of the black basses (*Micropterus* and *Huro*) with descriptions of four new forms. *Miscellaneous Publications Museum of Zoology University of Michigan*, **4**, 1-51.

Hubbs CL (1955) Hybridization between fish species in nature. *Systematic Zoology*, **4**, 1-20.

Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN (2007) Barcoding universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, **7**, 544-548.

Kassler TW *et al.* (2002) Molecular and morphological analyses of the black basses: Implications for taxonomy and conservation. *American Fisheries Society Symposium*, **31**, 291-322.

Koppelman JB (1994) Hybridization between smallmouth bass, *Micropterus dolomieu*, and spotted bass, *M. punctulatus*, in the Missouri River System, Missouri. *Copia*, **1994**, 204-210.

Leary RF, Allendorf, Sage GK (1995) Hybridization and introgression between introduced and native fish. Pages 91-101 *in* G.E. Hall, editor. Managing muskies. American Fisheries Society, Symposium 15, Bethesda, Maryland.

Littrell BM, Lutz-Carrillo DJ, Bonner TH, Fries LT (2007) Status of an introgressed Guadalupe bass population in a central Texas stream. *North American Journal of Fisheries Management*, **27**, 785-791.

Long, JM, Martin C (2008) The evolution of fisheries management of the upper Chattahoochee River in response to changing water management. Pages 21-36 in MS Allen, S Sammons, and MJ Maceina, Editors. Balancing Fisheries Management and water uses for impounded river systems. American Fisheries Society, Symposium 62, Bethesda, Maryland.

Lutz-Carrillo DJ, Hagen C, Dueck LA, Glenn TC (2008) Isolation and characterization of microsatellite loci for Florida largemouth bass, Micropter*us salmoides floridanus*, and other micropterids. *Molecular Ecology Resources*, **8**, 178-184.

Maceina MJ, Johnston CE, Stormer DG (2007) Distribution, abundance, biology and habitat use of shoal bass and species associates in selected tributaries of the Chattahoochee River, Alabama. Final report for Alabama Division of Wildlife and Freshwater Fisheries.

Martinsen GD, Whitham TG, Turek RJ, Keim P (2001) Hybrid populations selectively filter gene introgression between species. *Evolution*, **55**, 1325-1335.

Mettee MF, O'Neil PE, Pierson JM (1996) *Fishes of Alabama and the Mobile Basin.* Oxmoor House, Birmingham, Alabama.

Mirarchi RE, Garner JT, Mettee MF, O'Neil PE (eds) 2004. Alabama wildlife, volume two: Imperiled aquatic mollusks and fishes. The University of Alabama Press, Montgomery.

Morizot DC, Calhoun SW, Clepper LL, Schmidt ME (1991) Multispecies hybridization among native and introduced Centrarchid basses in Central Texas. *Transactions of the American Fisheries Society*, **120**, 283-289.

Pierce PC, Van Den Avyle MJ (1997) Hybridization between introduced spotted bass and smallmouth bass in reservoirs. *Transactions of the American Fisheries Society*, **126**, 939-947.

Pipas JC, Bulow FJ (1998) Hybridization between redeye bass and smallmouth bass in Tennessee streams. *Transactions of the American Fisheries Society*, **127**, 141-146.

Perry WL, Lodge DM, Feder JL (2002) Importance of hybridization between indigenous

and nonindigenous freshwater species: An overlooked threat to North American biodiversity. *Systematic Biology*, **51**, 255-275.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure from multilocus genotype data. *Genetics*, **155**, 945-959.

Pritchard JK, Wen W (2004) Documentation for structure software. Ver 2. http://pritch.bsd.uchicago.edu.

Ramsey JS, Smitherman RO (1972) Observations on spawning and growth of four species of basses (*Micropterus*) in ponds. *Proceedings of the Associated Fish and Wildlife Agencies*, **25**, 357-365.

Ratnasingham S, Hebert PDN (2007) BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes*, **7**, 355–364. DOI: 10.1111/j.1471-8286.2006.01678.x.

Rawson PD, Hilbish TJ (1998) Asymmetric introgression of mitochondrial DNA among European populations of blue mussels (*Mytilus* spp.). *Evolution*, **52**,100-108.

Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics*, **27**, 83-109.

Sammons SM, Maceina MJ (2009) Conservation status of shoal bass in Alabama: distribution, abundance, stocking efficacy, and possible effects of sympatric congeneric black bass in selected tributaries of the Chattahoochee River, Alabama. Final report submitted to Alabama Division of Wildlife and Freshwater Fisheries, Montgomery.

Trigali, M., W. Porak, P. Bellotti, S. Seyoum, N. Trippel, A. Strickland, J. Knight, C. Paxton, C. Puchulutegui, and M. David. 2010. Population Genetics of Freshwater Fishes for Fish Management and Stock Enhancement. Completion report for the Federal Aid in Sport Fish Restoration Act Grant F-128 to the U.S. Department of Interior Fish and Wildlife Conservation Commission. 60 pp.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of Biological Sciences*, **360**, 1847-1857.

Warm Springs Conservation Genetics Lab (2011) Standard operating procedure for collecting tissue samples for genetic analysis.

Whitmore DH (1983) Introgressive hybridization of smallmouth bass (*Micropterus dolomieu*) and Guadalupe bass (*M. treculi*). *Copia*, **1983**, 672-679.

Williams JD, Burgess GH (1999) A new species of bass, *Micropterus cataractae*

(Teleostei: Centrarchidae), from the Apalachicola River basin in Alabama, Florida, and Georgia. *Bulletin of the Florida Museum of Natural History*, **42**, 80-114.

Williams JE, Johnson JF, Hendrickson DA, Contreras-Balderas S, Williams JD, Navarro-Mendoza M, McAllister DE, Deacon JE (1989) Fishes of North America endangered, threatened, or of special concern. *Fisheries*, **14**, 2-20.

Wolf DE, Takebayashi N, Rieseberg LH (2001) Predicting the risks of extinction through hybridization. *Conservation Biology*, **15**, 1039-1053.

Zalapa JE, Brunet J, Guries RP (2010) The extent of hybridization and its impact on the genetic diversity and population structure of an invasive tree, *Ulmus pumila* (Ulmaceae). *Evolutionary Applications* **3**, 157-168.

Table 1. Characterization of 4 microsatellite loci for *Micropterus* specimens (N = 130) from the Chattahoochee River between Columbus, GA and Phenix City, AL. N represents the number of samples analyzed and N_A represents the number of alleles present per locus.

Locus	Primer Sequence (5'- 3')	Repeat Motif	Size Range (bp)	N	N_A
Msa-06	F:GACAGTGCACCAGGCCAAG R:ATCTGCAGGAGATTCTAGAGGATG	$(AC)_{13}$	96-118	129	10
Msa-10	F:ATCCCTCTCCCTCACTCTCTCTAT R:AAACTGTTTGAAATCTTTTGTTCCA	$(CA)_{19}$	110-152	127	13
Msa-22	F:CCGAGCAGGGCAGCAGGAGAGGCAAG R:ACTTTATGTCTGAAGAGCAGTGACA	$(CA)_{16}$	150-183	129	11
Msa-32	F:CCCCTTCATCAGATTTTATATGGTT R:AGGTCACATGCTGACTTTGTTACAC	$(AC)_{13}$	257-303	127	18

Table 2. Admixture coefficients (q), as determined by STRUCTURE, for *Micropterus* specimens (N = 130) found within the Chattahoochee River between Columbus, GA and Phenix City, AL.

Sample ID	Admixture Coefficient (q)	
	Shoal Bass	Spotted Bass
DB_T2_2	0.005	0.995
DB_T2_3	0.006	0.994
DB_T2_4	0.008	0.992
DB_T2_5	0.007	0.993
DB_T2_6	0.005	0.995
DB_T2_7	0.006	0.994
DB_T2_9	0.011	0.989
DB_T2_10	0.006	0.994
DB_T2_11	0.011	0.989
DB_T2_12	0.010	0.990
DB_T2_13	0.008	0.992
DB_T2_14	0.006	0.994
DB_T3_1	0.005	0.995
DB_T3_4	0.008	0.992
DB_T3_7	0.007	0.993
DB_T3_8	0.006	0.994
DB_T4_2	0.009	0.991
DB_T4_4	0.008	0.992
DB_T4_5	0.005	0.995
DB_T4_12	0.008	0.992
DB_T4_14	0.022	0.978
DB_T5_1	0.011	0.989
DB_T6_4	0.006	0.994
DB_T6_8	0.006	0.994
DB_T6_10	0.008	0.992
DB_T8_1	0.006	0.994
DB_T8_2	0.008	0.992
DB_T8_5	0.995	0.005
DB_T8_6	0.995	0.005
DB_T8_7	0.994	0.006
DB_T8_9	0.010	0.990
DB_T9_2	0.987	0.013
DB_T9_3	0.006	0.994
DB_T11_1	0.006	0.994
DB_T11_2	0.009	0.991
DB_T13_1	0.006	0.994
DB_T13_2	0.093	0.907

DB_T13_5	0.984	0.016
DB_T13_6	0.010	0.990
DB_T13_7	0.005	0.995
DB_T13_8	0.981	0.019
DB_T13_9	0.992	0.008
EP_T1_2	0.007	0.993
EP_T1_3	0.009	0.991
EP_T2_1	0.006	0.994
EP_T3_3	0.006	0.994
EP_T3_4	0.006	0.994
EP_T4_1	0.992	0.008
EP_T7_1	0.007	0.993
CM_T2_8	0.009	0.991
CM_T3_1	0.988	0.012
CM_T4_2	0.006	0.994
CM_T4_4	0.014	0.986
CM_T4_5	0.008	0.992
CM_T4_6	0.007	0.993
CM_T4_7	0.027	0.973
CM_T5_4	0.995	0.005
DB2_T2_5	0.006	0.994
DB2_T2_6	0.006	0.994
DB2_T2_7	0.007	0.993
DB2_T2_8	0.007	0.993
DB2_T2_9	0.007	0.993
DB2_T2_10	0.006	0.994
DB2_T2_11	0.007	0.993
DB2_T2_12	0.011	0.989
DB2_T2_13	0.087	0.913
DB2_T2_14	0.006	0.994
DB2_T2_15	0.010	0.990
DB2_T5_1	0.012	0.988
DB2_T5_2	0.005	0.995
DB2_T5_3	0.006	0.994
DB2_T5_4	0.007	0.993
DB2_T5_5	0.006	0.994
EP2_T1_1	0.010	0.990
EP2_T7_1	0.010	0.990
EP2_T8_1	0.009	0.991
EP2_T8_2	0.008	0.992
CM2_T1_3	0.008	0.992
CM2_T3_1_sb	0.008	0.992
CM2_T3_2_sb	0.994	0.006
CM2_T3_3_sb	0.005	0.995
CM2_T3_4_sb	0.007	0.993

CM2_T3_5_sb	0.990	0.010
CM2_T3_6_sb	0.006	0.994
CM2_T3_7_sb	0.006	0.994
CM2_T4_1_sb	0.007	0.993
CM2_T4_2_sb	0.009	0.991
CM2_T4_3_sb	0.995	0.005
CM2_T5_1	0.995	0.005
CM2_T5_2	0.007	0.993
CM2_T5_3	0.007	0.993
CM2_T6_1	0.007	0.993
CM2_T6_3	0.014	0.986
CM2_T6_4	0.006	0.994
CM2_T6_8	0.012	0.988
DB_T2_1	0.011	0.989
DB_T2_8	0.027	0.973
DB_T3_2	0.100	0.900
DB_T3_5	0.988	0.012
DB_T3_9	0.010	0.990
DB_T8_4	0.994	0.006
DB_T8_8	0.994	0.006
EP_T1_12	0.978	0.022
EP_T1_14	0.996	0.004
CM_T4_17	0.011	0.989
EP2_T3_1	0.975	0.025
DB_T3_3	0.011	0.989
DB_T4_3	0.012	0.988
DB_T4_6	0.025	0.975
DB_T4_7	0.994	0.006
DB_T4_8	0.007	0.993
DB_T4_9	0.994	0.006
DB_T4_10	0.026	0.974
DB_T4_13	0.115	0.885
DB_T6_5	0.024	0.976
DB_T6_6	0.014	0.986
DB_T6_7	0.206	0.794
DB_T6_9	0.980	0.020
DB_T8_3	0.991	0.009
DB_T13_3	0.992	0.008
EP_T1_4	0.012	0.988
EP_T1_9	0.058	0.942
EP_T3_2	0.346	0.654
EP_T3_20	0.013	0.987
EP_T4_3	0.151	0.849
EP_T5_6	0.991	0.009
EP_T6_4	0.990	0.010

EP2_T3_2	0.984	0.016
EP2_6_6	0.620	0.380
CM2_T5_1	0.971	0.029

Table 3. Allele frequencies for *Micropterus cataractae* and *M. punctulatus* found within the Chattahoochee River between Columbus, GA and Phenix City, AL. Numbers in parentheses are the number of genotypes used in the calculation. Highlighted frequencies are those with the most common alleles of that species.

Locus	*M. cataractae*	*M. punctulatus*
Msa-06		
Allele	(30)	(99)
96	0.009	0.046
98	0.248	0.444
100	0.262	0.002
102	0.173	0.078
104	0.029	0.368
106	0.008	0.005
108	0.009	0.046
112	0.020	0.001
116	0.122	0.008
118	0.121	0.001
Msa-10		
Allele	(30)	(97)
110	0.020	0.001
124	0.092	0.001
126	0.007	0.026
128	0.035	0.001
130	0.204	0.316
132	0.094	0.158
134	0.392	0.008
136	0.092	0.001
138	0.007	0.021
140	0.022	0.304
146	0.007	0.016
150	0.018	0.034
152	0.009	0.113

Msa-22 Allele	(30)	(99)
150	0.009	0.010
151	0.013	0.629
159	0.078	0.187
161	0.036	0.001
167	0.340	0.165
171	0.235	0.002
173	0.093	0.001
177	0.021	0.001
179	0.107	0.001
181	0.035	0.001
183	0.035	0.001

Msa-32 Allele	(29)	(98)
257	0.007	0.056
263	0.072	0.020
267	0.035	0.001
269	0.006	0.026
271	0.009	0.283
273	0.007	0.041
275	0.011	0.055
281	0.035	0.256
283	0.034	0.001
285	0.035	0.001
287	0.094	0.069
289	0.309	0.002
291	0.104	0.076
293	0.119	0.096
297	0.020	0.001
299	0.006	0.016
301	0.064	0.001
303	0.034	0.001

Table 4. Summary of P values for Chi-Square tests of independence between and among the three putative groups of *Micropterus* as determined by microsatellite analysis.

Characteristic	Shoal-Spotted	Shoal-Hybrid	Spotted-Hybrid
Shallow Dorsal Notch	7.39E-05	0.09	0.08
Mouth Terminus Below Eye	8.86E-04	0.10	1
Tooth Patch Present	3.80E-07	0.38	2.35E-04
Lateral Spots Present	2.16E-25	0.69	1.62E-30

Table 5. Comparison of morphological, microsatellite (with three separate cutoffs to hybridity), and barcoding identification of *Micropterus* specimens (N = 35) from the Chattahoochee River between Columbus, GA and Phenix City, AL. LMB represents *M. salmoides*, largemouth bass.

Sample ID	Morph ID	Micro ID (0.95)	Micro ID (0.983)	Micro ID (0.99)	Barcode ID
DB_13_3	Shoal	Shoal	Shoal	Shoal	Shoal
DB_3_5	Hybrid	Shoal	Shoal	Hybrid	Shoal
CM_5_4	Spotted	Shoal	Shoal	Shoal	Florida
DB_8_6	Spotted	Shoal	Shoal	Shoal	Florida
DB_8_5	Spotted	Shoal	Shoal	Shoal	LMB
DB_8_7	Spotted	Shoal	Shoal	Shoal	LMB
DB_9_2	Spotted	Shoal	Shoal	Hybrid	LMB
CM2_3_5	Spotted	Shoal	Shoal	Hybrid	Florida
CM2_4_3	Spotted	Shoal	Shoal	Shoal	Florida
DB_6_9	Shoal	Shoal	Hybrid	Hybrid	LMB
DB_4_6	Shoal	Spotted	Hybrid	Hybrid	Redeye
DB_4_13	Shoal	Hybrid	Hybrid	Hybrid	Spotted
DB_6_5	Shoal	Spotted	Hybrid	Hybrid	Spotted
DB_6_7	Shoal	Hybrid	Hybrid	Hybrid	Redeye
EP_4_3	Shoal	Hybrid	Hybrid	Hybrid	Shoal
DB_4_10	Shoal	Spotted	Hybrid	Hybrid	Spotted
EP_1_9	Shoal	Hybrid	Hybrid	Hybrid	Redeye
EP_3_2	Shoal	Hybrid	Hybrid	Hybrid	LMB
DB_2_8	Hybrid	Spotted	Hybrid	Hybrid	Spotted
DB_3_2	Hybrid	Hybrid	Hybrid	Hybrid	Spotted
DB_3_3	Shoal	Spotted	Spotted	Hybrid	Redeye
DB_2_1	Hybrid	Spotted	Spotted	Hybrid	Redeye
CM_4_4	Spotted	Spotted	Spotted	Hybrid	Redeye
CM_4_5	Spotted	Spotted	Spotted	Spotted	Redeye
DB_13_7	Spotted	Spotted	Spotted	Spotted	Redeye
DB_2_12	Spotted	Spotted	Spotted	Hybrid	Redeye
DB_2_2	Spotted	Spotted	Spotted	Spotted	Redeye
DB_2_13	Spotted	Spotted	Spotted	Spotted	Redeye
DB_2_3	Spotted	Spotted	Spotted	Spotted	Redeye
DB_2_4	Spotted	Spotted	Spotted	Spotted	Redeye
DB_2_5	Spotted	Spotted	Spotted	Spotted	Redeye
DB_3_1	Spotted	Spotted	Spotted	Spotted	Redeye
DB_2_14	Spotted	Spotted	Spotted	Spotted	Redeye
CM2_6_3	Spotted	Spotted	Spotted	Hybrid	Redeye
DB2_5_1	Spotted	Spotted	Spotted	Hybrid	Redeye

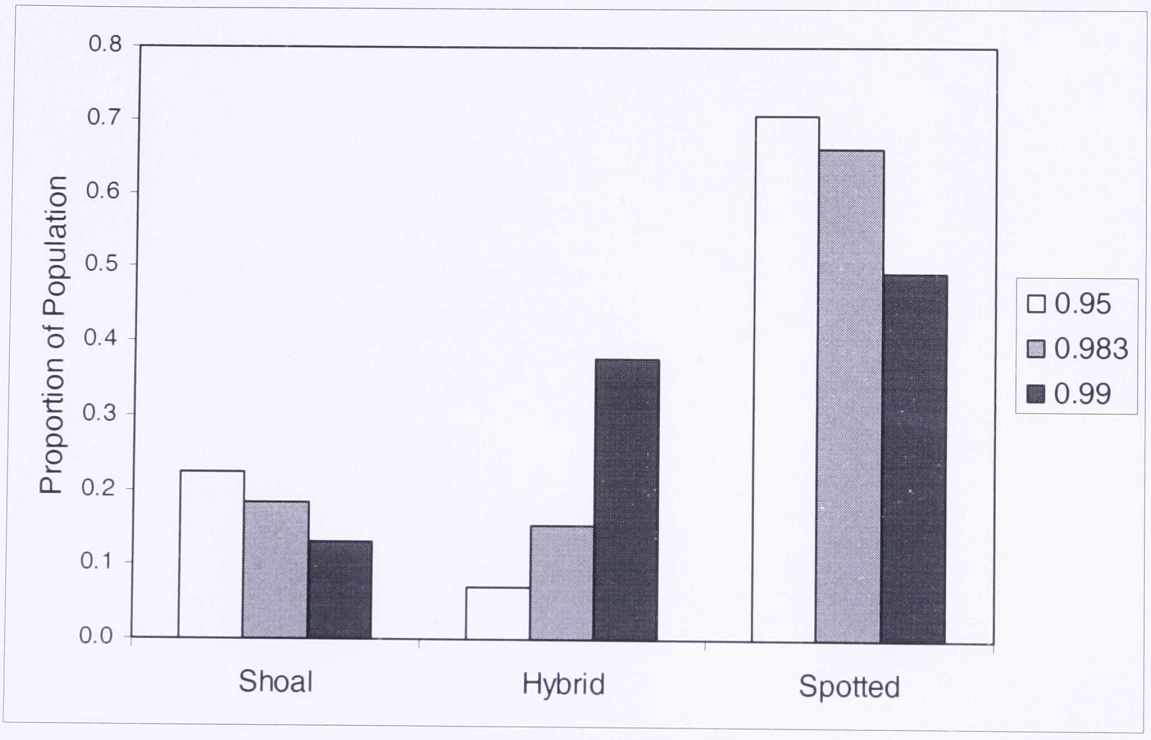

Figure 1. The proportion of shoal, hybrid, and spotted bass present in the sample population (N = 130) as determined by microsatellite analysis comparing $q > 0.95$, 0.983, 0.99 as cutoffs to hybridity.

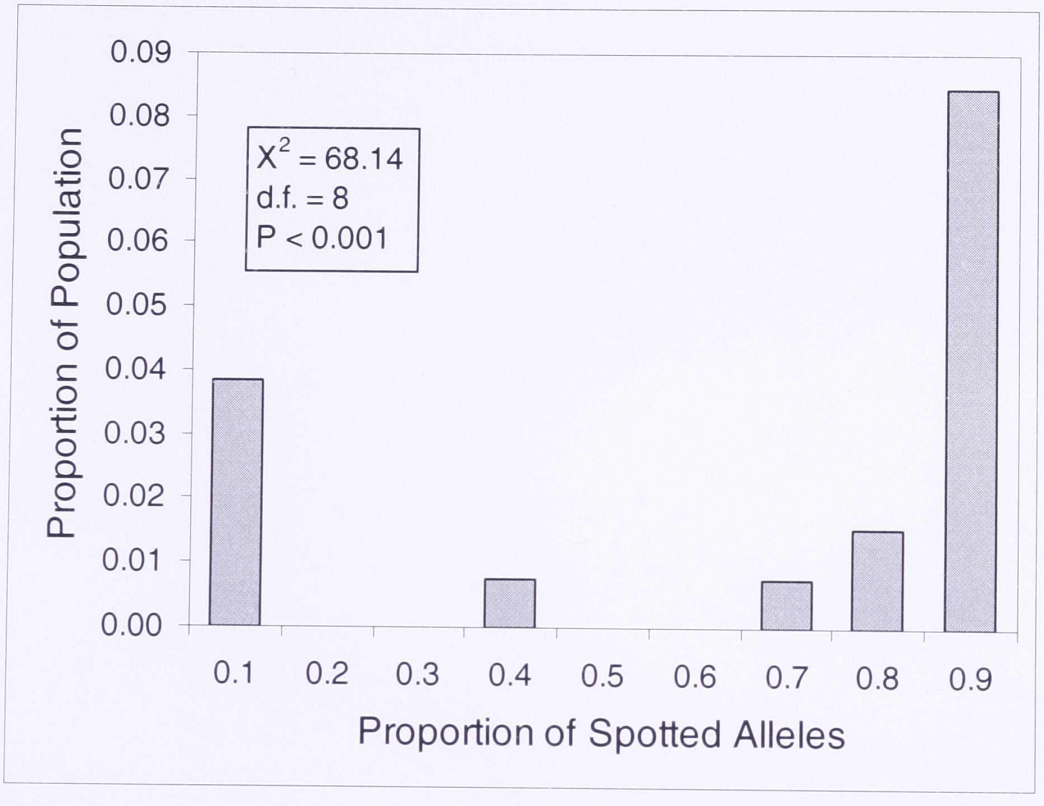

Figure 2. The proportion of spotted alleles present in shoal x spotted hybrids (N = 20) as determined by microsatellite analysis using $q > 0.983$ as the cutoff to hybridity.

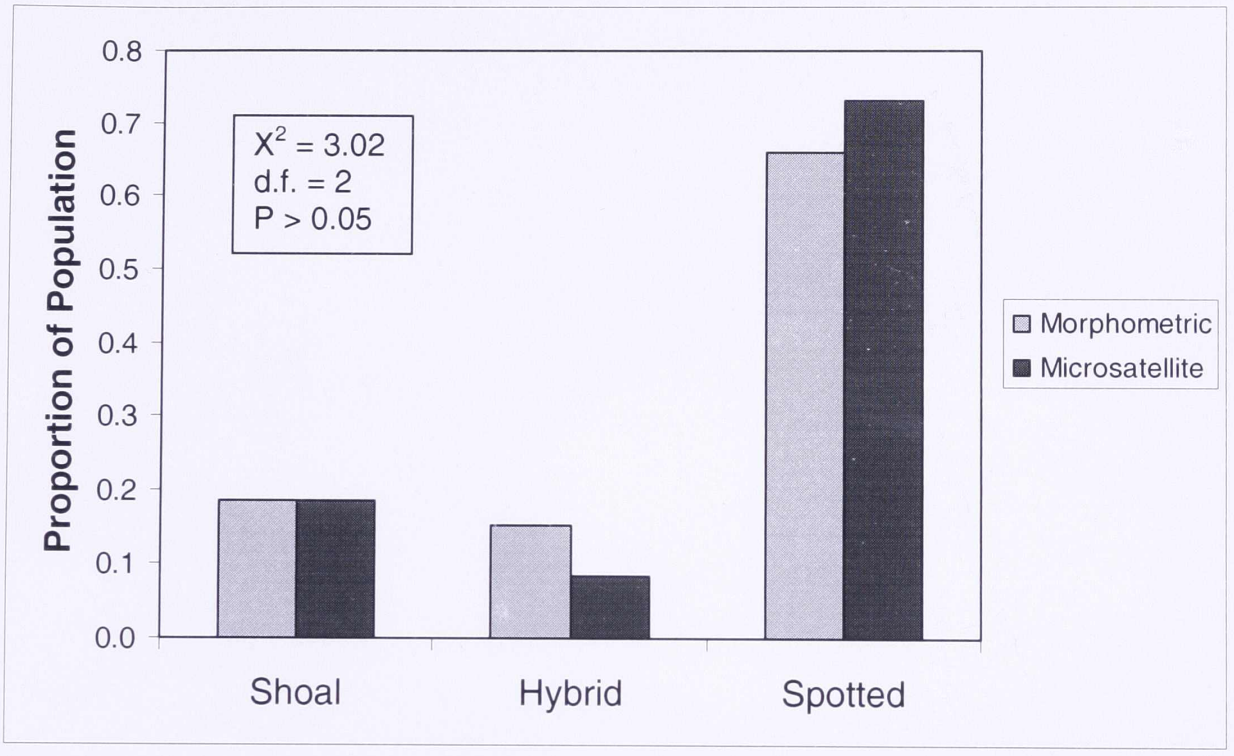

Figure 3. A comparison between the proportion of shoal, hybrid, and spotted bass as determined by morphometrics and microsatellite analyses.

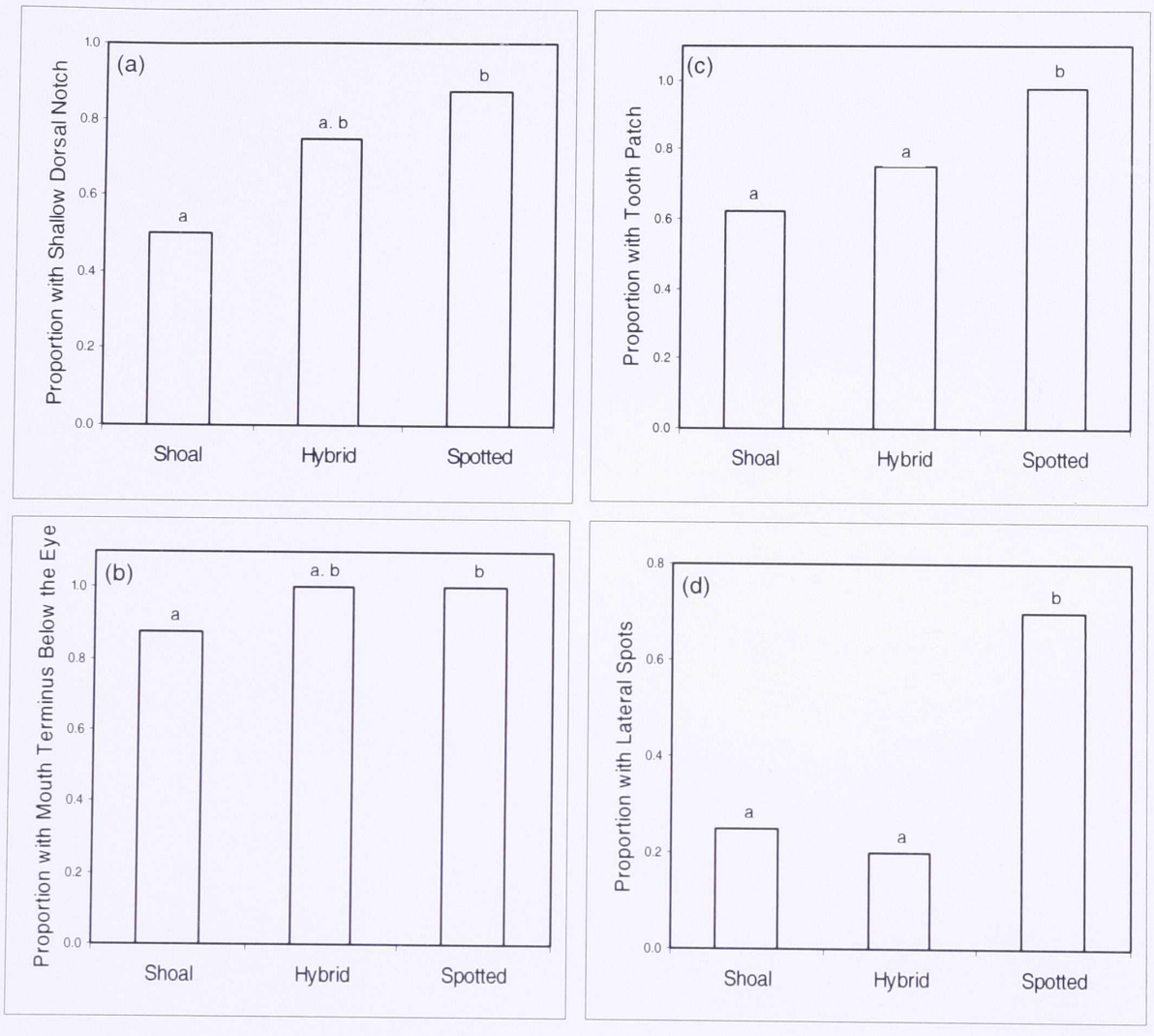

Figure 4. The proportion of shoal, hybrid, and spotted bass as determined by microsatellites with the following structure based characteristics: (a) shallow dorsal notch; (b) mouth terminus extending below the eye; (c) presence of a tooth patch; (d) presence of lateral spots. Letters represent significant difference.

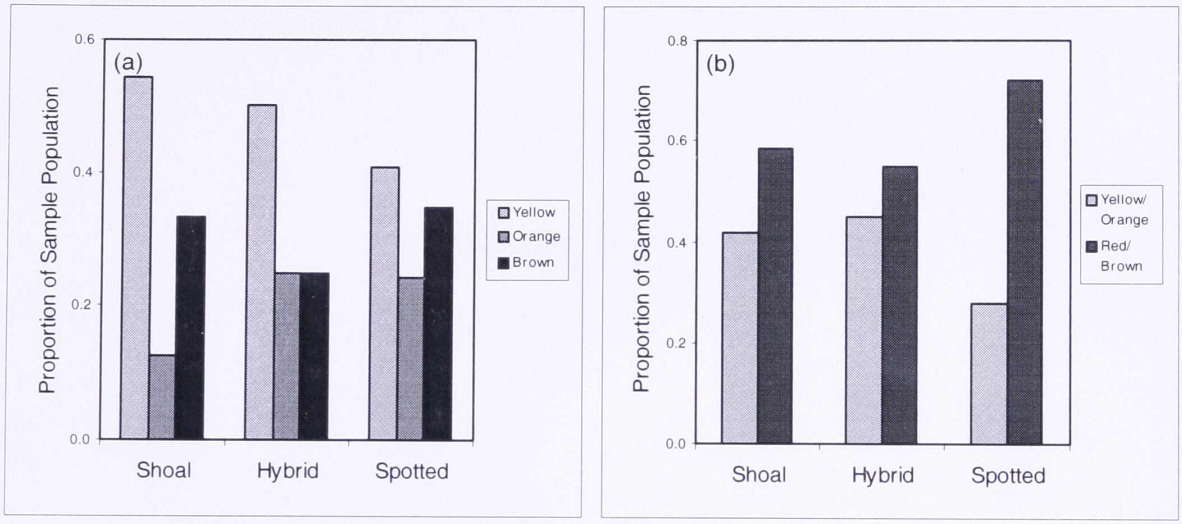

Figure 5. The proportion of shoal, hybrid, and spotted bass as determined by microsatellites with the following color based characteristics: (a) caudal fin color; (b) eye color.

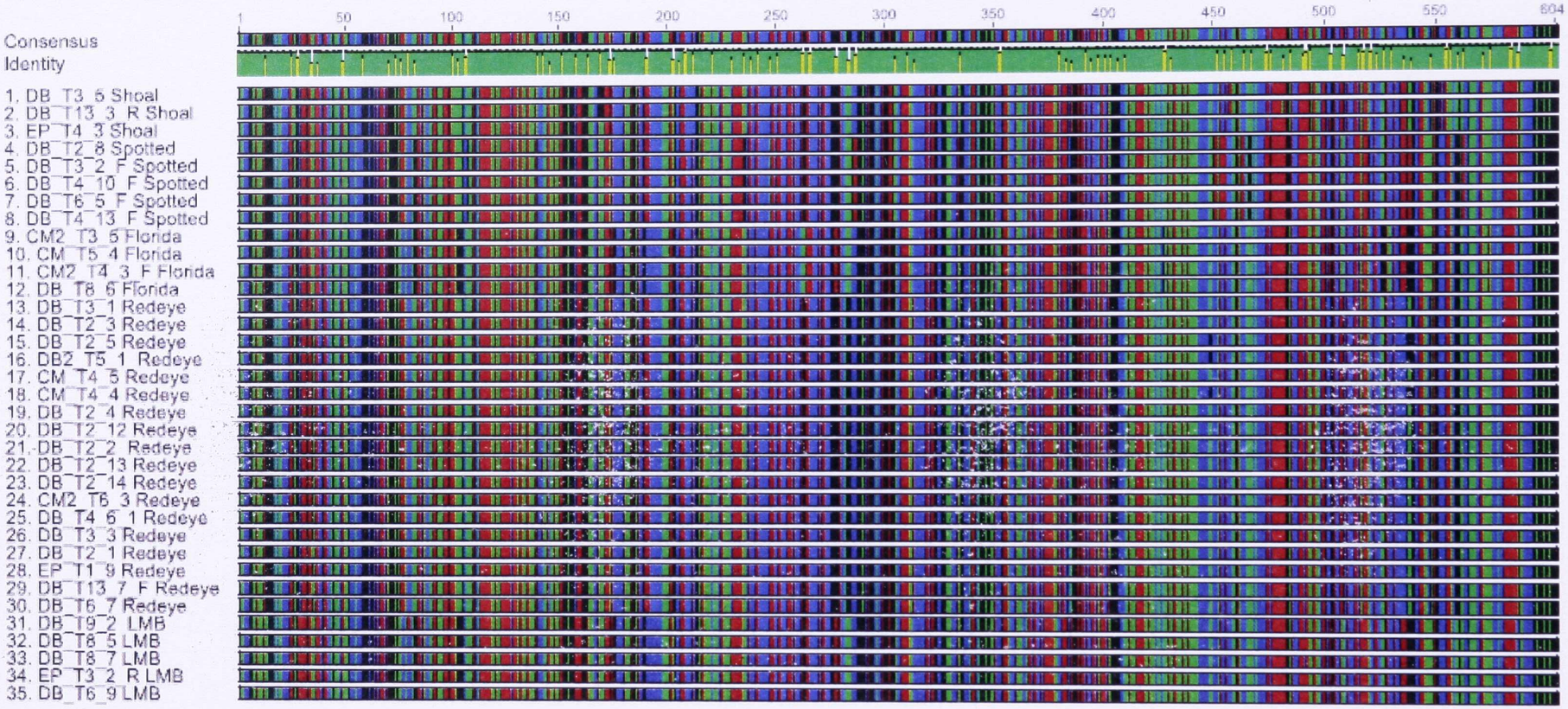

Figure 6. COI sequence alignment (604 bp) for *Micropterus* species [shoal, spotted, Florida, redeye, largemouth bass] (N = 35) found within the Chattahoochee River between Columbus, GA and Phenix City, AL.

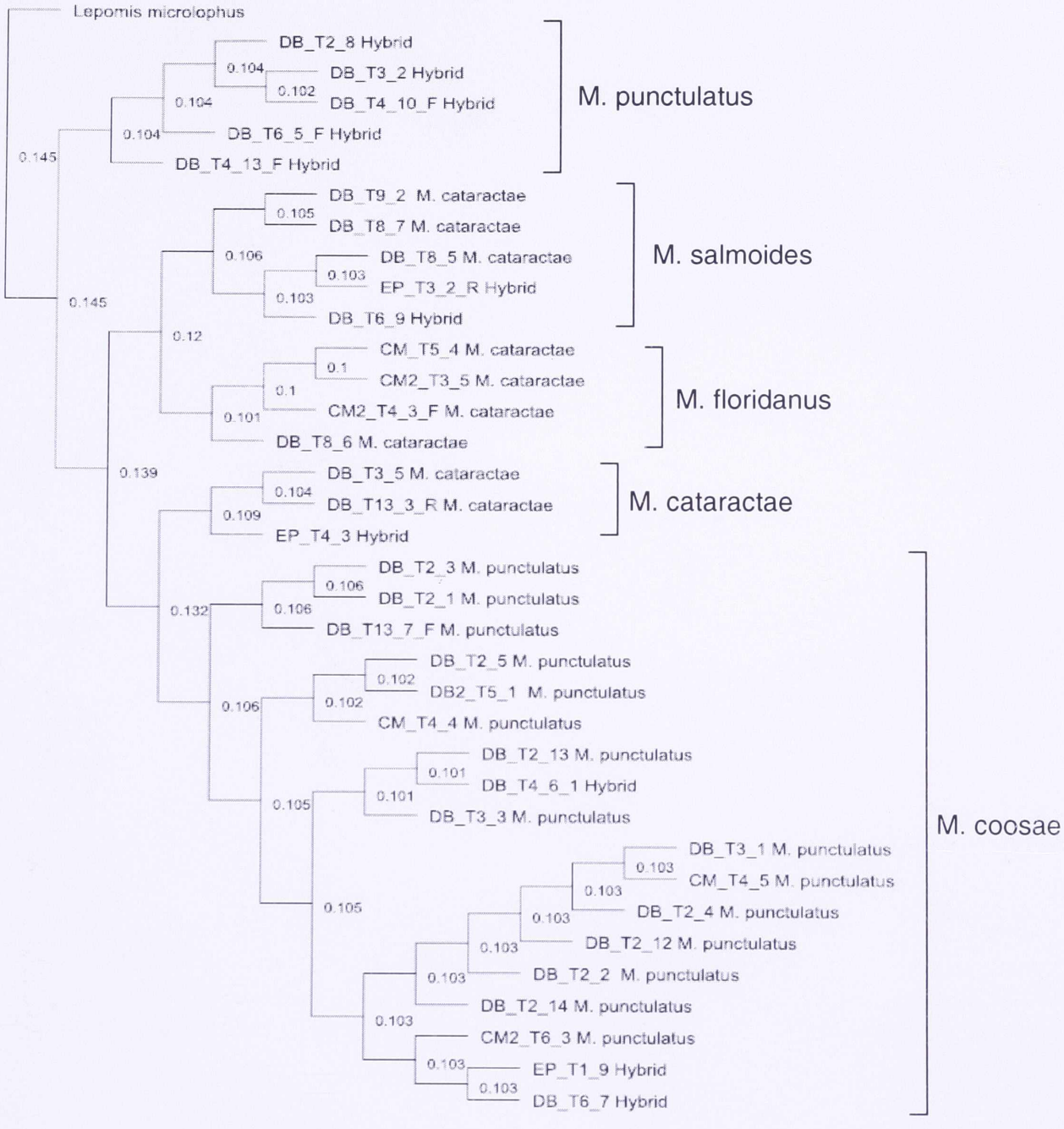

Figure 7. Neighbor-joining tree for *Micropterus* species (N = 35), as determined by microsatellite analysis, found within the Chattahoochee River between Columbus, GA and Phenix City, AL. Scientific names enclosed in brackets indicate BLASTn result identities ≥ 99.5%. The tree was rooted using *Lepomis microlophus* (redear sunfish) as an outgroup.

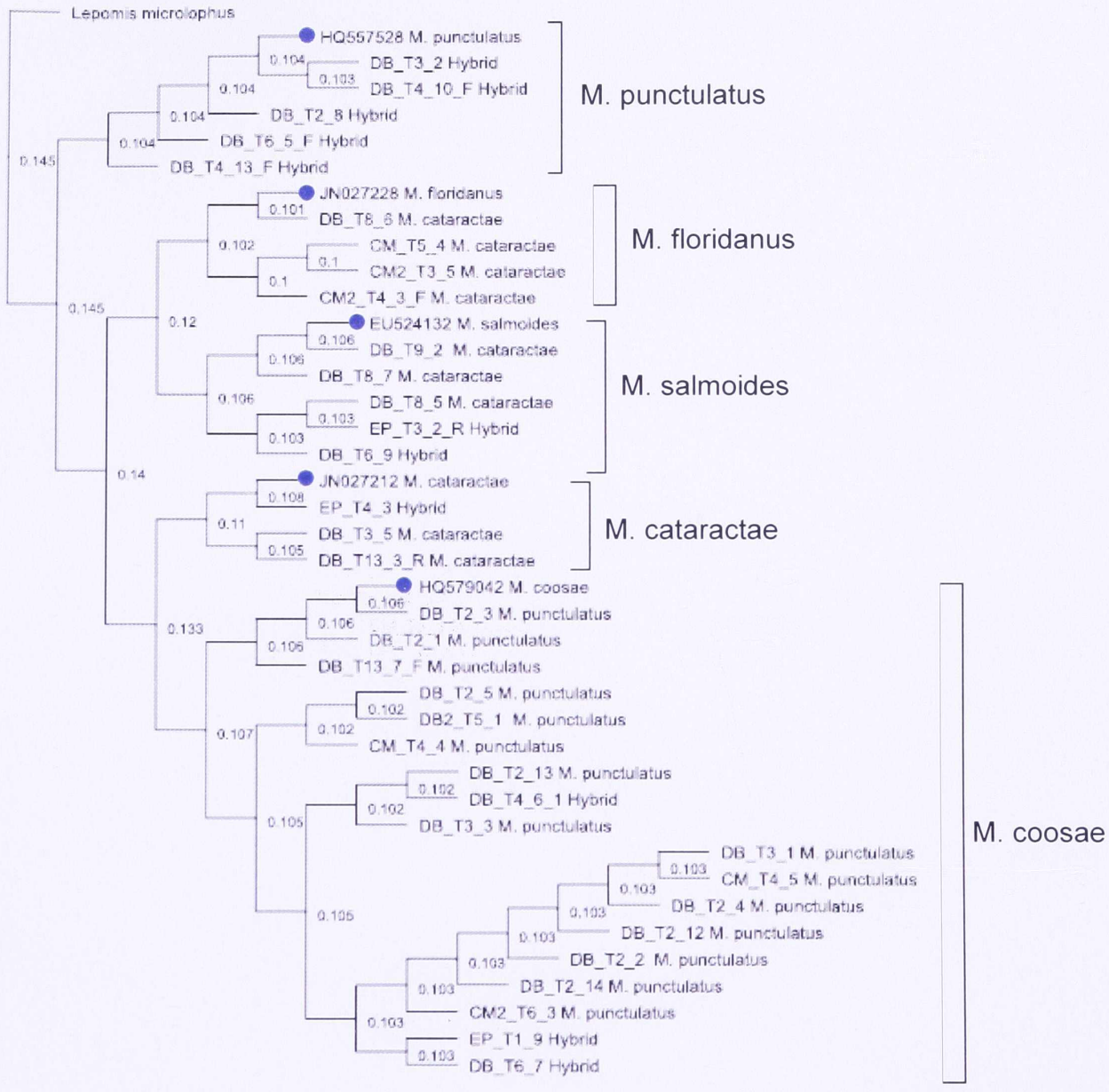

Figure 8. Neighbor-joining tree for Micropterus species (N = 35), as determined by microsatellite analysis, found within the Chattahoochee River between Columbus, GA and Phenix City, AL. Scientific names enclosed in brackets indicated BLASTn result indentities ≥ 99.5%. Samples with blue dots indicate reference samples of each parental species from GenBank. The tree was rooted using Lemois microlophus (redear sunfish) as an outgroup.

APPENDIX A

Morphological data for *Micropterus* specimens (N = 130) found within the Chattahoochee River between Columbus, GA and Phenix City, AL.

Sample ID	Morph ID	Season	Teeth	Mouth Terminus	Dorsal Notch	Black Spots	Caudal Color	Eye Color
DB_13_3	Shoal	Fall	Absent	Below	Shallow	Absent	Orange	Orange
DB_4_10	Shoal	Fall	Present	Below	Shallow	Absent	Yellow	Orange
DB_4_13	Shoal	Fall	Present	Below	Deep	Absent	Yellow	Brown
DB_4_3	Shoal	Fall	Present	Below	Shallow	Present	Yellow	Brown
DB_4_6	Shoal	Fall	Present	Below	Shallow	Absent	Yellow	Brown
DB_4_7	Shoal	Fall	Present	Below	Deep	Absent	Yellow	Brown
DB_4_8	Shoal	Fall	Present	Below	Shallow	Absent	Yellow	Brown
DB_4_9	Shoal	Fall	Absent	Below	Deep	Absent	Yellow	Brown
DB_6_5	Shoal	Fall	Present	Below	Shallow	Present	Yellow	Brown
DB_6_6	Shoal	Fall	Present	Below	Shallow	Present	Yellow	Brown
DB_6_7	Shoal	Fall	Present	Below	Shallow	Present	Yellow	Brown
DB_6_9	Shoal	Fall	Present	Below	Deep	Absent	Yellow	Yellow
DB_8_3	Shoal	Fall	Present	Below	Shallow	Absent	Yellow	Brown
EP_1_4	Shoal	Fall	Present	Below	Shallow	Absent	Yellow	Brown
EP_1_9	Shoal	Fall	Present	Below	Shallow	Absent	Orange	Orange
EP_3_2	Shoal	Fall	Absent	Below	Shallow	Absent	Brown	Red
EP_3_20	Shoal	Fall	Present	Below	Deep	Present	Yellow	Yellow
EP_4_3	Shoal	Fall	Absent	Below	Shallow	Absent	Brown	Brown
EP_5_6	Shoal	Fall	Absent	Below	Shallow	Absent	Yellow	Yellow
EP_6_4	Shoal	Fall	Present	Below	Deep	Absent	Brown	Orange
CM2_5_1_Sh	Shoal	Spring	Absent	Below	Shallow	Absent	Yellow	Brown
EP2_3_2	Shoal	Spring	Absent	Below	Shallow	Absent	Brown	Brown
EP2_6_6	Shoal	Spring	Absent	Below	Shallow	Absent	Brown	Brown
DB_3_3	Shoal	Fall	Present	Below	Shallow	Absent	Yellow	Orange
CM_4_17	Hybrid	Fall	Present	Below	Shallow	Absent	Yellow	Orange
DB_2_1	Hybrid	Fall	Present	Below	Shallow	Absent	Orange	Brown
DB_2_8	Hybrid	Fall	Present	Below	Shallow	Absent	Orange	Orange
DB_3_2	Hybrid	Fall	Present	Below	Shallow	Absent	Yellow	Yellow
DB_3_5	Hybrid	Fall	Absent	Below	Shallow	Absent	Brown	Brown
DB_3_9	Hybrid	Fall	Present	Below	Shallow	Present	Brown	Brown
DB_8_4	Hybrid	Fall	Present	Below	Deep	Absent	Yellow	Orange
DB_8_8	Hybrid	Fall	Present	Below	Deep	Absent	Yellow	Orange
EP_1_12	Hybrid	Fall	Absent	Below	Deep	Absent	Yellow	Yellow
EP_1_14	Hybrid	Fall	Absent	Below	Deep	Absent	Yellow	Orange
EP2_3_1	Hybrid	Spring	Present	Below	Shallow	Absent	Orange	Yellow
CM_2_8	Spotted	Fall	Present	Below	Deep	Present	Brown	Orange
CM_3_1	Spotted	Fall	Absent	Below	Shallow	Present	Brown	Brown
CM_4_2	Spotted	Fall	Present	Below	Shallow	Present	Brown	Red
CM_4_4	Spotted	Fall	Present	Below	Deep	Present	Brown	Brown

CM_4_5	Spotted	Fall	Present	Below	Shallow	Present	Brown	Brown
CM_4_6	Spotted	Fall	Present	Below	Shallow	Present	Brown	Brown
CM_4_7	Spotted	Fall	Present	Below	Shallow	Present	Brown	Brown
CM_5_4	Spotted	Fall	Present	Below	Deep	Absent	Brown	Brown
DB_11_1	Spotted	Fall	Present	Below	Shallow	Absent	Orange	Yellow
DB_11_2	Spotted	Fall	Present	Below	Shallow	Absent	Orange	Yellow
DB_13_1	Spotted	Fall	Present	Below	Shallow	Present	Yellow	Yellow
DB_13_2	Spotted	Fall	Present	Below	Shallow	Absent	Orange	Brown
DB_13_5	Spotted	Fall	Present	Below	Deep	Absent	Orange	Yellow
DB_13_6	Spotted	Fall	Present	Below	Shallow	Absent	Orange	Brown
DB_13_7	Spotted	Fall	Present	Below	Shallow	Absent	Orange	Brown
DB_13_8	Spotted	Fall	Present	Below	Shallow	Absent	Orange	Orange
DB_13_9	Spotted	Fall	Present	Below	Shallow	Absent	Orange	Brown
DB_2_10	Spotted	Fall	Present	Below	Shallow	Absent	Orange	Yellow
DB_2_11	Spotted	Fall	Present	Below	Shallow	Absent	Yellow	Brown
DB_2_12	Spotted	Fall	Absent	Below	Shallow	Absent	Yellow	Brown
DB_2_13	Spotted	Fall	Present	Below	Shallow	Absent	Yellow	Brown
DB_2_2	Spotted	Fall	Present	Below	Shallow	Present	Yellow	Brown
DB_2_3	Spotted	Fall	Present	Below	Shallow	Absent	Orange	Red
DB_2_4	Spotted	Fall	Present	Below	Shallow	Absent	Orange	Red
DB_2_5	Spotted	Fall	Present	Below	Shallow	Absent	Orange	Red
DB_2_6	Spotted	Fall	Present	Below	Shallow	Absent	Orange	Brown
DB_2_7	Spotted	Fall	Present	Below	Shallow	Absent	Orange	Orange
DB_2_9	Spotted	Fall	Present	Below	Shallow	Absent	Orange	Orange
DB_3_1	Spotted	Fall	Present	Below	Shallow	Absent	Yellow	Yellow
DB_3_4	Spotted	Fall	Present	Below	Shallow	Present	Yellow	Brown
DB_3_7	Spotted	Fall	Present	Below	Shallow	Present	Brown	Brown
DB_3_8	Spotted	Fall	Present	Below	Shallow	Present	Brown	Brown
DB_4_12	Spotted	Fall	Present	Below	Shallow	Absent	Yellow	Brown
DB_4_14	Spotted	Fall	Present	Below	Deep	Absent	Yellow	Yellow
DB_4_2	Spotted	Fall	Present	Below	Shallow	Present	Yellow	Brown
DB_4_4	Spotted	Fall	Present	Below	Shallow	Present	Yellow	Red
DB_4_5	Spotted	Fall	Present	Below	Shallow	Present	Yellow	Orange
DB_5_1	Spotted	Fall	Present	Below	Shallow	Present	Yellow	Brown
DB_6_10	Spotted	Fall	Present	Below	Shallow	Present	Yellow	Brown
DB_6_4	Spotted	Fall	Present	Below	Shallow	Present	Yellow	Brown
DB_6_8	Spotted	Fall	Present	Below	Shallow	Absent	Yellow	Orange
DB_8_1	Spotted	Fall	Present	Below	Shallow	Present	Yellow	Brown
DB_8_2	Spotted	Fall	Present	Below	Shallow	Present	Yellow	Brown
DB_8_5	Spotted	Fall	Present	Below	Deep	Absent	Yellow	Orange
DB_8_6	Spotted	Fall	Present	Below	Shallow	Absent	Yellow	Brown
DB_8_7	Spotted	Fall	Present	Below	Shallow	Absent	Yellow	Orange
DB_8_9	Spotted	Fall	Present	Below	Shallow	Absent	Orange	Brown
DB_9_2	Spotted	Fall	Absent	Below	Deep	Absent	Yellow	Yellow
DB_9_3	Spotted	Fall	Present	Below	Shallow	Absent	Orange	Yellow
EP_1_2	Spotted	Fall	Present	Below	Deep	Present	Brown	Brown
EP_1_3	Spotted	Fall	Present	Below	Shallow	Present	Yellow	Brown
EP_2_1	Spotted	Fall	Present	Below	Shallow	Present	Yellow	Brown

EP_3_3	Spotted	Fall	Present	Below	Shallow	Present	Brown	Orange
EP_3_4	Spotted	Fall	Present	Below	Shallow	Present	Brown	Orange
EP_4_1	Spotted	Fall	Absent	Behind	Shallow	Present	Brown	Brown
EP_7_1	Spotted	Fall	Present	Below	Shallow	Present	Yellow	Orange
DB_2_14	Spotted	Fall	Absent	Below	Shallow	Absent	Orange	Brown
CM2_1_3	Spotted	Spring	Present	Below	Deep	Present	Brown	Brown
CM2_3_1	Spotted	Spring	Present	Below	Shallow	Present	Yellow	Brown
CM2_3_2	Spotted	Spring	Present	Behind	Deep	Present	Brown	Brown
CM2_3_3	Spotted	Spring	Present	Below	Shallow	Present	Yellow	Brown
CM2_3_4	Spotted	Spring	Present	Below	Shallow	Present	Yellow	Brown
CM2_3_5	Spotted	Spring	Present	Behind	Shallow	Present	Brown	Brown
CM2_3_6	Spotted	Spring	Present	Below	Deep	Present	Brown	Brown
CM2_3_7	Spotted	Spring	Present	Below	Shallow	Present	Brown	Brown
CM2_4_1	Spotted	Spring	Present	Below	Shallow	Present	Yellow	Brown
CM2_4_2	Spotted	Spring	Present	Below	Shallow	Present	Brown	Brown
CM2_4_3	Spotted	Spring	Present	Below	Deep	Present	Yellow	Brown
CM2_5_1	Spotted	Spring	Present	Below	Shallow	Present	Yellow	Brown
CM2_5_2	Spotted	Spring	Present	Below	Shallow	Present	Brown	Brown
CM2_5_3	Spotted	Spring	Present	Below	Shallow	Present	Brown	Orange
CM2_6_1	Spotted	Spring	Present	Below	Shallow	Absent	Orange	Yellow
CM2_6_3	Spotted	Spring	Present	Below	Shallow	Present	Orange	Brown
CM2_6_4	Spotted	Spring	Present	Below	Shallow	Present	Brown	Brown
CM2_6_8	Spotted	Spring	Present	Below	Shallow	Present	Brown	Yellow
DB2_2_10	Spotted	Spring	Present	Below	Shallow	Present	Brown	Brown
DB2_2_11	Spotted	Spring	Present	Below	Deep	Present	Brown	Brown
DB2_2_12	Spotted	Spring	Present	Below	Shallow	Present	Yellow	Brown
DB2_2_13	Spotted	Spring	Present	Below	Deep	Present	Brown	Brown
DB2_2_14	Spotted	Spring	Present	Below	Deep	Present	Yellow	Brown
DB2_2_15	Spotted	Spring	Present	Below	Deep	Present	Orange	Brown
DB2_2_5	Spotted	Spring	Present	Below	Shallow	Present	Brown	Brown
DB2_2_6	Spotted	Spring	Present	Below	Shallow	Present	Yellow	Brown
DB2_2_7	Spotted	Spring	Present	Below	Deep	Present	Orange	Brown
DB2_2_8	Spotted	Spring	Present	Below	Shallow	Present	Brown	Brown
DB2_2_9	Spotted	Spring	Present	Below	Shallow	Present	Yellow	Brown
DB2_5_1	Spotted	Spring	Present	Below	Shallow	Present	Brown	Brown
DB2_5_2	Spotted	Spring	Present	Below	Shallow	Present	Brown	Orange
DB2_5_3	Spotted	Spring	Present	Below	Shallow	Present	Brown	Orange
DB2_5_4	Spotted	Spring	Present	Below	Shallow	Present	Brown	Yellow
DB2_5_5	Spotted	Spring	Present	Below	Shallow	Present	Brown	Brown
EP2_1_1	Spotted	Spring	Present	Below	Shallow	Present	Orange	Brown
EP2_7_1	Spotted	Spring	Present	Below	Shallow	Present	Brown	Yellow
EP2_8_1	Spotted	Spring	Present	Below	Deep	Present	Orange	Brown
EP2_8_2	Spotted	Spring	Present	Below	Shallow	Present	Brown	Brown

APPENDIX B

Microsatellite data for *Micropterus* specimens (N = 190) found within the Chattahoochee River between Columbus, GA and Phenix City, AL. Numbers shaded grey indicate lack of data collected.

Sample ID	Morph ID	Msa-06		Msa-10		Msa-22		Msa-32	
DB_T3_3	Shoal	104	108	138	140	167	167	271	281
DB_T4_3	Shoal	98	104	126	130	167	167	271	271
DB_T4_6	Shoal	98	104	140	150	150	150	271	281
DB_T4_7	Shoal	98	100	124	136	171	171	263	267
DB_T4_8	Shoal	98	104	132	146	159	159	271	281
DB_T4_9	Shoal	98	100	134	136	171	171	263	289
DB_T4_10	Shoal	98	104	140	150	159	167	263	281
DB_T4_13	Shoal	102	102	130	150	159	167	271	293
DB_T6_5	Shoal	102	104	130	140	159	167	281	287
DB_T6_6	Shoal	98	104	126	130	167	167	271	271
DB_T6_7	Shoal	102	102	130	140	167	167	281	291
DB_T6_9	Shoal	100	100	134	134	173	183	281	293
DB_T8_3	Shoal	118	118	130	130	167	167	289	289
DB_T13_3	Shoal	112	116	130	134	167	167	289	293
EP_T1_4	Shoal	98	108	140	150	159	167	281	299
EP_T1_9	Shoal	98	104	130	132	167	167	275	287
EP_T3_2	Shoal	98	116	132	140	167	167	275	287
EP_T3_20	Shoal	98	98	140	140	167	167	271	281
EP_T4_3	Shoal	98	116	132	140	167	167	271	281
EP_T5_6	Shoal	116	118	110	132	167	167	289	293
EP_T6_4	Shoal	98	116	130	134	161	167	287	289
EP2_T3_2	Shoal	98	118	130	130	161	167	287	289
EP2_T6_6	Shoal	98	116	140	150	167	167	281	289
CM2_T5_1	Shoal	98	116	132	132	167	167	289	291
DB_T2_1	Hybrid	98	108	130	150	159	167	271	271
DB_T2_8	Hybrid	98	98	146	150	167	167	271	281
DB_T3_2	Hybrid	98	104	130	130	167	167	263	281
DB_T3_5	Hybrid	98	118	130	134	167	167	287	289
DB_T3_9	Hybrid	104	104	130	140	167	167	271	281
DB_T8_4	Hybrid	98	100	134	136	173	179	263	289
DB_T8_8	Hybrid	98	100	128	134	179	179	291	303
EP_T1_12	Hybrid	100	104	134	136	171	173	283	297
EP_T1_14	Hybrid	100	100	134	136	171	179	283	289
CM_T4_17	Hybrid	96	108	132	140	167	167	271	281
EP2_T3_1	Hybrid	98	116	130	132	167	167	287	289
DB_T2_2	Spotted	104	104	140	152	151	159	271	275
DB_T2_3	Spotted	96	96	130	132	151	159	271	271
DB_T2_4	Spotted	98	102	130	140	151	151	275	281

DB_T2_5	Spotted	104	104	130	140	0	0	271	281
DB_T2_6	Spotted	104	108	130	140	151	159	271	281
DB_T2_7	Spotted	98	98	140	140	151	151	257	269
DB_T2_9	Spotted	98	104	130	138	151	151	263	271
DB_T2_10	Spotted	98	104	130	138	151	151	281	281
DB_T2_11	Spotted	98	104	130	138	151	151	263	271
DB_T2_12	Spotted	104	104	130	140	159	159	293	293
DB_T2_13	Spotted	98	104	130	132	151	159	271	291
DB_T2_14	Spotted	98	104	140	152	151	151	281	293
DB_T3_1	Spotted	104	104	130	152	151	151	271	281
DB_T3_4	Spotted	98	102	130	130	151	151	271	271
DB_T3_7	Spotted	98	102	132	140	151	151	271	273
DB_T3_8	Spotted	98	98	140	140	151	151	281	281
DB_T4_2	Spotted	98	104	130	132	151	159	271	291
DB_T4_4	Spotted	98	98	130	146	151	151	281	299
DB_T4_5	Spotted	98	104	140	140	151	151	271	281
DB_T4_12	Spotted	104	104	130	152	151	151	291	293
DB_T4_14	Spotted	98	104	130	152	159	167	287	293
DB_T5_1	Spotted	98	102	0	0	151	151	281	281
DB_T6_4	Spotted	98	104	132	140	151	159	271	281
DB_T6_8	Spotted	98	98	130	152	151	151	271	281
DB_T6_10	Spotted	98	98	132	140	151	151	281	291
DB_T8_1	Spotted	98	104	132	140	151	151	271	275
DB_T8_2	Spotted	98	98	132	140	151	167	271	281
DB_T8_5	Spotted	98	100	124	134	171	171	267	289
DB_T8_6	Spotted	100	100	134	134	171	183	289	293
DB_T8_7	Spotted	98	100	134	136	171	171	263	289
DB_T8_9	Spotted	104	104	130	132	151	159	287	293
DB_T9_2	Spotted	102	102	134	134	159	179	291	291
DB_T9_3	Spotted	104	104	130	132	151	159	271	271
DB_T11_1	Spotted	104	104	132	152	151	151	271	293
DB_T11_2	Spotted	98	104	132	140	151	151	293	293
DB_T13_1	Spotted	96	108	130	152	151	159	271	275
DB_T13_2	Spotted	98	104	130	134	151	151	281	287
DB_T13_5	Spotted	100	102	130	134	159	181	291	293
DB_T13_6	Spotted	98	102	130	132	151	151	275	281
DB_T13_7	Spotted	98	104	140	140	151	151	271	299
DB_T13_8	Spotted	98	102	134	134	159	177	0	0
DB_T13_9	Spotted	98	100	128	134	179	181	293	293
EP_T1_2	Spotted	98	98	126	140	151	151	281	293
EP_T1_3	Spotted	96	102	132	140	151	151	271	291
EP_T2_1	Spotted	104	104	130	130	151	159	257	271
EP_T3_3	Spotted	98	104	130	140	151	151	257	257
EP_T3_4	Spotted	98	104	140	152	151	151	273	293
EP_T4_1	Spotted	118	118	130	134	167	167	287	289
EP_T7_1	Spotted	104	104	130	152	151	159	271	291
CM_T2_8	Spotted	104	104	130	130	159	159	271	291
CM_T3_1	Spotted	116	118	130	132	167	167	289	291

CM_T4_2	Spotted	96	98	132	140	151	151	281	281
CM_T4_4	Spotted	102	104	130	130	151	159	273	291
CM_T4_5	Spotted	98	104	126	126	151	159	257	287
CM_T4_6	Spotted	98	98	130	140	151	151	281	293
CM_T4_7	Spotted	98	106	130	152	151	159	281	287
CM_T5_4	Spotted	100	102	124	134	171	173	289	301
DB2_T2_5	Spotted	98	98	130	140	151	151	271	281
DB2_T2_6	Spotted	98	98	140	140	151	151	273	281
DB2_T2_7	Spotted	98	98	140	140	151	159	269	293
DB2_T2_8	Spotted	98	98	140	152	151	151	269	291
DB2_T2_9	Spotted	98	98	140	140	151	151	281	293
DB2_T2_10	Spotted	0	0	140	152	151	151	281	281
DB2_T2_11	Spotted	98	108	130	132	151	151	271	293
DB2_T2_12	Spotted	98	98	130	130	151	167	257	257
DB2_T2_13	Spotted	98	98	134	140	151	151	273	293
DB2_T2_14	Spotted	98	98	140	152	151	151	269	281
DB2_T2_15	Spotted	98	108	0	0	151	151	269	293
DB2_T5_1	Spotted	102	102	130	140	151	151	281	281
DB2_T5_2	Spotted	104	104	130	130	151	151	257	271
DB2_T5_3	Spotted	98	98	140	152	151	151	281	281
DB2_T5_4	Spotted	104	104	130	130	159	159	271	273
DB2_T5_5	Spotted	98	98	152	152	151	151	257	257
EP2_T1_1	Spotted	98	102	140	152	151	159	271	287
EP2_T7_1	Spotted	104	104	130	132	151	151	291	291
EP2_T8_1	Spotted	98	104	140	152	151	167	271	293
EP2_T8_2	Spotted	98	104	130	132	151	151	275	291
CM2_T1_3	Spotted	96	104	132	152	151	159	0	0
CM2_T3_1	Spotted	104	104	130	132	151	159	271	287
CM2_T3_2	Spotted	102	102	130	134	171	179	301	303
CM2_T3_3	Spotted	98	104	132	152	151	151	271	271
CM2_T3_4	Spotted	98	104	130	132	151	159	257	275
CM2_T3_5	Spotted	98	102	124	134	159	171	285	285
CM2_T3_6	Spotted	104	108	130	132	151	159	271	281
CM2_T3_7	Spotted	98	98	140	140	151	159	275	275
CM2_T4_1	Spotted	98	104	130	140	151	151	271	291
CM2_T4_2	Spotted	104	104	0	0	151	151	0	0
CM2_T4_3	Spotted	100	102	124	134	171	173	289	301
CM2_T5_1	Spotted	100	102	124	134	171	173	289	301
CM2_T5_2	Spotted	96	98	132	152	151	151	281	291
CM2_T5_3	Spotted	96	104	130	132	151	151	271	287
CM2_T6_1	Spotted	98	104	140	140	151	167	271	273
CM2_T6_3	Spotted	102	104	130	130	151	159	273	287
CM2_T6_4	Spotted	98	104	130	140	151	159	271	281
CM2_T6_8	Spotted	98	104	130	132	151	151	287	287
DB_T3_6	LMB	100	102	124	134	173	179	267	269
DB_T3_10	LMB	118	118	132	132	167	167	287	289
DB_T4_1	LMB	98	100	134	134	173	175	263	291
DB_T6_1	LMB	100	100	128	134	167	183	267	287

DB_T6_2	LMB	100	100	132	136	167	171	263	293
DB_T6_3	LMB	100	100	134	134	159	171	263	285
EP_T1_1	LMB	98	100	122	132	159	173	285	289
EP_T5_1	LMB	118	118	132	132	167	167	289	291
CM_T1_1	LMB	98	98	128	136	159	173	289	293
CM_T1_2	LMB	100	100	134	134	171	179	289	305
CM_T1_3	LMB	0	0	134	134	171	179	301	303
CM_T1_4	LMB	100	104	134	134	171	183	293	313
CM_T1_5	LMB	100	100	124	128	173	179	269	291
CM_T1_6	LMB	100	110	124	138	159	183	267	285
CM_T2_7	LMB	100	104	134	134	173	181	293	305
CM_T2_10	LMB	100	102	128	128	171	173	289	307
CM_T2_13	LMB	100	100	134	134	171	179	259	305
CM_T3_2	LMB	100	104	134	134	179	183	267	281
CM_T3_3	LMB	116	118	130	132	167	167	287	289
CM_T3_4	LMB	116	118	130	132	167	167	289	289
CM_T4_1	LMB	98	102	128	134	159	159	261	293
CM_T4_3	LMB	98	100	128	134	179	183	287	297
CM_T5_1	LMB	100	100	134	134	171	173	291	293
CM_T5_2	LMB	98	102	128	134	159	159	263	301
CM_T5_3	LMB	100	100	132	134	171	173	291	293
CM_T5_6	LMB	100	100	134	134	171	171	261	267
DB2_T2_3	LMB	100	100	128	128	159	179	0	0
DB2_T2_4	LMB	100	102	124	138	159	197	301	301
EP2_T5_1	LMB	100	100	124	134	179	181	285	293
EP2_T6_1	LMB	100	110	116	128	169	183	0	0
EP2_T6_3	LMB	98	102	128	134	159	179	261	293
EP2_T6_4	LMB	98	110	128	132	169	179	0	0
EP2_T6_5	LMB	100	104	124	134	159	179	289	289
EP2_T7_2	LMB	100	100	112	128	173	175	269	283
EP2_T7_3	LMB	100	102	134	134	159	183	303	303
EP2_T8_3	LMB	100	100	124	134	159	171	259	267
EP2_T8_4	LMB	100	110	128	134	171	173	285	291
CM2_T1_1	LMB	98	100	128	134	179	183	0	0
CM2_T1_2	LMB	100	100	128	136	159	173	0	0
CM2_T1_4	LMB	100	100	134	138	171	179	0	0
CM2_T1_5	LMB	104	110	134	134	171	171	0	0
CM2_T1_6	LMB	100	100	134	138	171	173	281	289
CM2_T1_7	LMB	100	100	134	134	171	183	293	293
CM2_T1_8	LMB	98	100	134	138	179	183	291	301
CM2_T1_9	LMB	100	102	124	134	159	173	0	0
CM2_T1_10	LMB	100	100	128	128	173	175	289	303
CM2_T3_2_lmb	LMB	98	98	130	132	167	167	0	0
CM2_T5_1_lmb	LMB	100	104	112	134	171	183	261	303
CM2_T5_2_lmb	LMB	98	100	136	136	171	171	291	291
CM2_T6_2	LMB	100	104	112	136	171	171	285	289
CM2_T6_5	LMB	100	100	128	128	169	173	285	293
CM2_T6_6	LMB	100	100	128	128	171	171	263	263

CM2_T6_7	LMB	100	100	128	134	159	183	285	291
CM2_T6_9	LMB	100	100	134	134	171	173	291	293
CM2_T6_10	LMB	100	104	134	134	179	183	285	291
CM2_T6_11	LMB	100	110	136	138	173	179	267	291
CM2_T6_12	LMB	100	100	124	134	179	181	267	299
CM2_T6_13	LMB	100	100	124	128	173	179	269	269
CM2_T6_14	LMB	100	102	134	134	159	179	0	0
DB_T9_1	LMB	98	100	132	136	171	179	291	291

APPENDIX C

mtDNA sequences of *Micropterus* specimens (N = 35) found within the Chattahoochee River between Columbus, GA and Phenix City, AL.

***Micropterus punctulatus* (n = 5)**

DB_T4_13_COI-F *Micropterus punctulatus*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTAATTCGTGCAGAACTAAG
CCAGCCCGGCGCTCTTCTAGGGGATGACCAGATCTACAATGTAATTGTTACA
GCGCATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATTATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATAGCATTCC
CTCGAATAAACAACATAAGCTTTTGGCTTCTTCCCCCATCTTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAAGCTGGAGCTGGCACTGGGTGAACTGTCTACCC
CCCTCTTGCCGGCAACCTGGCCCATGCAGGAGCATCCGTTGACCTAACCATCT
TCTCTCTTCATCTTGCGGGTGTCTCCTCCATCCTAGGGGCCATCAATTTTATTA
CCACAATTATTAATATAAAACCCCCAGCTATTTCCCAGTATCAGACACCCTTG
TTTGTTTGGTCCGTCTTAATTACTGCCGTCCTACTCCTTTTATCGCTCCCAGTC
CTCGCTGCTGGCATTACAATGCTCCTTACGGATCGAAACCTCAACACCACCTT
CTTTGACCCCGCAGGAGGGGG

DB_T4_10_COI-F *Micropterus punctulatus*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTAATTCGTGCAGAACTAAG
CCAGCCCGGCGCTCTTCTAGGGGATGACCAGATCTACAATGTAATTGTTACA
GCGCATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATTATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATAGCATTTC
CTCGAATAAACAACATAAGCTTTTGGCTTCTTCCCCCATCTTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAAGCTGGAGCTGGCACTGGGTGAACTGTCTACCC
CCCTCTTGCCGGCAACCTGGCCCATGCAGGAGCATCCGTTGACCTAACCATCT
TCTCTCTTCATCTTGCGGGTGTCTCCTCCATCCTAGGGGCCATCAATTTTATTA
CCACAATTATTAATATAAAACCCCCAGCTATTTCCCAGTATCAGACACCCTTG
TTTGTTTGGTCCGTCTTAATTACTGCCGTCCTACTCCTTTTATCGCTCCCAGTC
CTCGCTGCTGGCATTACAATGCTCCTTACGGATCGAAACCTCAACACCACCTT
CTTTGACCCCGCAGGAGGGGG

DB_T6_5_COI-F *Micropterus punctulatus*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTAATTCGTGCAGAACTAAG
CCAGCCCGGCGCTCTTCTAGGGGATGACCAGATCTACAATGTAATTGTTACA
GCGCATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATTATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATAGCATTCC
CTCGAATAAACAACATAAGCTTTTGGCTTCTTCCCCCATCTTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAAGCTGGAGCTGGCACTGGGTGAACTGTCTACCC
CCCTCTTGCCGGCAACCTGGCCCATGCAGGAGCATCCGTTGACCTAACCATCT
TCTCTCTTCATCTTGCGGGTGTCTCCTCCATCCTAGGGGCCATCAATTTTATTA
CCACAATTATTAATATAAAACCCCCAGCTATTTCCCAGTATCAGACACCCTTG

TTTGTTTGGTCCGTCTTAATTACTGCCGTCCTACTCCTTTTATCGCTCCCAGTC
CTCGCTGCTGGCATTACAATGCTCCTTACGGATCGAAACCTCAACACCACCTT
CTTTGACCCCGCAGGAGGGGG

DB_T3_2_COI-F *Micropterus punctulatus*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTAATTCGTGCAGAACTAAG
CCAGCCCGGCGCTCTTCTAGGGGATGACCAGATCTACAATGTAATTGTTACA
GCGCATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATTATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATAGCATTTC
CTCGAATAAACAACATAAGCTTTTGGCTTCTTCCCCCATCTTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAAGCTGGAGCTGGCACTGGGTGAACTGTCTACCC
CCCTCTTGCCGGCAACCTGGCCCATGCAGGAGCATCCGTTGACCTAACCATCT
TCTCTCTTCATCTTGCGGGTGTCTCCTCCATCCTAGGGGCCATCAATTTTATTA
CCACAATTATTAATATAAAACCCCCAGCTATTTCCCAGTATCAGACACCCTTG
TTTGTTTGGTCCGTCTTAATTACTGCCGTCCTACTCCTTTTATCGCTCCCAGTC
CTCGCTGCTGGCATTACAATGCTCCTTACGGATCGAAACCTCAACACCACCTT
CTTTGACCCCGCAGGAGGGGG

DB_T2_8 *Micropterus punctulatus*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTAATTCGTGCAGAACTAAG
CCAGCCCGGCGCTCTTCTAGGGGATGACCAGATCTACAATGTAATTGTTACA
GCGCATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATTATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATAGCATTCC
CTCGAATAAACAACATAAGCTTTTGGCTTCTTCCCCCATCTTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAAGCTGGAGCTGGCACTGGGTGAACTGTCTACCC
CCCTCTTGCCGGCAACCTGGCCCATGCAGGAGCATCCGTTGACCTAACCATCT
TCTCTCTTCATCTTGCGGGTGTCTCCTCCATCCTAGGGGCCATCAATTTTATTA
CCACAATTATTAATATAAAACCCCCAGCTATTTCCCAGTATCAGACACCCTTG
TTTGTTTGGTCCGTCTTAATTACTGCCGTCCTACTCCTTTTATCGCTCCCAGTC
CTCGCTGCTGGCATTACAATGCTCCTTACGGATCGAAACCTCAACACCACCTT
CTTTGACCCCGCAGGAGGGGG

***Micropterus salmoides* (n = 5)**

EP_T3_2_COI-R *Micropterus salmoides*
GAGCCGGAATAGTAGGCACAGCCCTGAGTCTGCTAATTCGTGCAGAACTAAG
CCAACCGGGCGCTCTTCTGGGAGACGACCAGATCTACAATGTAATTGTTACG
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATTATAATTGGAGG
TTTTGGTAACTGACTTGTGCCCCTAATGATCGGCGCCCCCGACATAGCATTCC
CTCGAATAAACAACATAAGTTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGTGTCGAAGCCGGGGCTGGCACTGGGTGGACTGTTTACCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCACCTTGCTGGTGTCTCCTCTATTCTAGGGGCAATCAATTTTATTAC
CACAATTATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTTCTAATTACTGCTGTCCTACTCCTTCTGTCACTTCCAGTCC

TCGCTGCGGGCATTACAATGCTCCTTACAGACCGAAACCTCAACACCACCTTC
TTCGACCCCGCAGGAGGAGG

DB_T9_2 *Micropterus salmoides*
GAGCCGGAATAGTAGGCACAGCCCTGAGTCTGCTAATTCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTGGGAGACGATCAGATCTACAATGTAATTGTTACG
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATTATAATTGGAGG
TTTTGGTAACTGACTTGTGCCCCTAATGATCGGCGCCCCCGACATAGCATTCC
CTCGAATAAACAACATAAGTTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGTGTCGAAGCCGGGGCTGGCACTGGGTGGACTGTTTACCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCACCTTGCTGGTGTCTCCTCTATTCTAGGGGCAATCAATTTTATTAC
CACAATTATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCTGTCCTACTCCTTCTGTCACTTCCAGTCC
TCGCTGCGGGCATTACAATGCTCCTTACAGACCGAAACCTCAACACCACCTTC
TTCGACCCCGCAGGAGGAGG

DB_T8_7 *Micropterus salmoides*
GAGCCGGAATAGTAGGCACAGCCCTGAGTCTGCTAATTCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTGGGAGACGACCAGATCTACAATGTAATTGTTACG
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATTATAATTGGAGG
TTTTGGTAACTGACTTGTGCCCCTAATGATCGGCGCCCCCGACATAGCATTCC
CTCGAATAAACAACATAAGTTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGTGTCGAAGCCGGGGCTGGCACTGGGTGGACTGTTTACCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCACCTTGCTGGTGTCTCCTCTATTCTAGGGGCAATCAATTTTATTAC
CACAATTATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCTGTCCTACTCCTTCTGTCACTTCCAGTCC
TCGCTGCGGGCATTACAATGCTCCTTACAGACCGAAACCTCAACACCACCTTC
TTCGACCCCGCAGGAGGAGG

DB_T8_5 *Micropterus salmoides*
GAGCCGGAATAGTAGGCACAGCCCTGAGTCTGCTAATTCGTGCAGAACTAAG
CCAACCGGGCGCTCTTCTGGGAGACGACCAGATCTACAATGTAATTGTTACG
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATTATAATTGGAGG
TTTTGGTAACTGACTTGTGCCCCTAATGATCGGCGCCCCCGACATAGCATTCC
CTCGAATAAACAACATAAGTTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGTGTCGAAGCCGGGGCTGGCACTGGGTGGACTGTTTACCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCACCTTGCTGGTGTCTCCTCTATTCTAGGGGCAATCAATTTTATTAC
CACAATTATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTTCTAATTACTGCTGTCCTACTCCTTCTGTCACTTCCAGTCC
TCGCTGCGGGCATTACAATGCTCCTTACAGACCGAAACCTCAACACCACCTTC
TTCGACCCCGCAGGAGGAGG

DB_T6_9 *Micropterus salmoides*
GAGCCGGAATAGTAGGCACAGCCCTGAGTCTGCTAATTCGTGCAGAACTAAG
CCAACCGGGCGCTCTTCTGGGAGACGACCAGATCTACAATGTAATTGTTACG
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATTATAATTGGAGG
TTTTGGTAACTGACTTGTGCCCCTAATGATCGGCGCCCCCGACATAGCATTCC
CTCGAATAAACAACATAAGTTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGTGTCGAAGCCGGGGCTGGCACTGGGTGGACTGTTTACCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCACCTTGCTGGTGTCTCCTCTATTCTAGGGGCAATCAATTTTATTAC
CACAATTATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTTCTAATTACTGCTGTCCTACTCCTTCTGTCACTTCCAGTCC
TCGCTGCGGGCATTACAATGCTCCTTACAGACCGAAACCTCAACACCACCTTC
TTCGACCCCCGCAGGAGGAGG

***Micropterus floridanus* (n = 4)**

CM2_T4_3_COI-F *Micropterus floridanus*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTAATTCGTGCAGAACTAAG
CCAACCGGGCGCTCTTCTGGGAGACGACCAGATCTACAATGTAATTGTTACG
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATTATAATTGGAGG
CTTTGGCAACTGACTTGTGCCCCTAATGATCGGCGCCCCCGACATGGCATTCC
CTCGAATAAACAACATAAGTTTTTGACTCCTCCCCCCTTCCTTCCTTCTCCTAC
TTGCCTCTTCCGGTGTCGAAGCCGGGGCTGGCACTGGGTGGACTGTTTACCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCAGTTGACCTAACCATCTT
CTCTCTTCATCTTGCTGGTGTTTCCTCTATTCTAGGGGCAATCAATTTTATTAC
CACGATTATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTTTGATCCGTTCTAATTACTGCTGTCCTACTCCTTCTATCACTTCCGGTAC
TCGCTGCGGGCATTACAATGCTCCTTACGGACCGAAACCTTAATACCACCTTC
TTCGACCCCCGCAGGAGGAGG

CM_T5_4 *Micropterus floridanus*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTAATTCGTGCAGAACTAAG
CCAACCGGGCGCTCTTCTGGGAGACGACCAGATCTACAATGTAATTGTTACG
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATTATAATTGGAGG
CTTTGGCAACTGACTTGTGCCCCTAATGATCGGCGCCCCCGACATGGCATTCC
CTCGAATAAACAACATAAGTTTTTGACTCCTCCCCCCTTCCTTCCTTCTCCTAC
TTGCCTCTTCCGGTGTCGAAGCCGGGGCTGGCACTGGGTGGACTGTTTACCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCAGTTGACCTAACCATCTT
CTCTCTTCATCTTGCTGGTGTTTCCTCTATTCTAGGGGCAATCAATTTTATTAC
CACGATTATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTTTGATCCGTTCTAATTACTGCTGTCCTACTCCTTCTATCACTTCCGGTAC
TCGCTGCGGGCATTACAATGCTCCTTACGGACCGAAACCTTAATACCACCTTC
TTCGACCCCCGCAGGAGGAGG

CM2_T3_5 *Micropterus floridanus*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTAATTCGTGCAGAACTAAG

CCAACCGGGCGCTCTTCTGGGAGACGACCAGATCTACAATGTAATTGTTACG
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATTATAATTGGAGG
CTTTGGCAACTGACTTGTGCCCCTAATGATCGGCGCCCCCGACATGGCATTCC
CTCGAATAAACAACATAAGTTTTTGACTCCTCCCCCCTTCCTTCCTTCTCCTAC
TTGCCTCTTCCGGTGTCGAAGCCGGGGCTGGCACTGGGTGGACTGTTTACCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCAGTTGACCTAACCATCTT
CTCTCTTCATCTTGCTGGTGTTTCCTCTATTCTAGGGGCAATCAATTTTATTAC
CACGATTATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTTTGATCCGTTCTAATTACTGCTGTCCTACTCCTTCTATCACTTCCGGTAC
TCGCTGCGGGCATTACAATGCTCCTTACGGACCGAAACCTTAATACCACCTTC
TTCGACCCCGCAGGAGGAGG

DB_T8_6 *Micropterus floridanus*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTAATTCGTGCAGAACTAAG
CCAACCGGGCGCTCTTCTGGGAGACGACCAGATCTACAATGTAATTGTTACG
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATTATAATTGGAGG
CTTTGGCAACTGACTTGTGCCCCTAATGATCGGCGCCCCCGACATGGCATTCC
CTCGAATAAACAACATAAGTTTTTGACTCCTCCCCCCTTCCTTCCTTCTCCTAC
TTGCCTCTTCCGGTGTCGAAGCCGGAGCTGGCACTGGGTGGACTGTTTACCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCAGTTGACCTAACCATCTT
CTCTCTTCATCTTGCTGGTGTTTCCTCTATTCTAGGGGCAATCAATTTTATTAC
CACGATTATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTTTGATCCGTTCTAATTACTGCTGTCCTACTCCTTCTATCACTTCCGGTAC
TCGCTGCGGGCATTACAATGCTCCTTACGGACCGAAACCTTAATACCACCTTC
TTCGACCCCGCAGGAGGAGG

***Micropterus cataractae* (n = 3)**

DB_T13_3_COI-R *Micropterus cataractae*
GAGCCGGAATAGTGGGCACAGCCCTAAGCCTGCTAATTCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTGGGAGATGACCAAATCTACAATGTAATTGTAACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATACCCATTATAATTGGAGG
CTTTGGCAACTGGCTTGTCCCCCTAATGATCGGTGCCCCCGACATAGCATTCC
CCCGAATAAATAACATAAGCTTTTGACTTCTTCCCCCCTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAAGCCGGAGCTGGCACTGGGTGAACTGTTTACCC
CCCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCT
TCTCTCTTCATCTTGCTGGTGTCTCCTCCATCCTAGGGGCCATCAATTTTATTA
CCACAATTATTAATATAAAACCCCCAGCCATCTCTCAGTACCAAACACCCCTC
TTTGTATGATCCGTTCTGATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTC
CTCGCTGCAGGCATTACGATGCTCTTAACAGACCGAAACCTTAACACCACCTT
TTTTGACCCCGCAGGAGGAGG

DB_T3_5 *Micropterus cataractae*
GAGCCGGAATAGTGGGCACAGCCCTAAGCCTGCTAATTCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTGGGAGATGACCAAATCTACAATGTAATTGTAACA

GCACATGCATTTGTAATAATTTTCTTTATAGTAATACCCATTATAATTGGAGG
CTTTGGCAACTGGCTTGTCCCCCTAATGATCGGTGCCCCCGACATAGCATTCC
CCCGAATAAATAACATAAGCTTTTGACTTCTTCCCCCCTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAAGCCGGAGCTGGCACTGGGTGAACTGTTTACCC
CCCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCT
TCTCTCTTCATCTTGCTGGTGTCTCCTCCATCCTAGGGGCCATCAATTTTATTA
CCACAATTATTAATATAAAACCCCCAGCCATCTCTCAGTACCAAACACCCCTC
TTTGTCTGATCCGTTCTGATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTC
CTCGCTGCAGGCATTACGATGCTCTTAACAGACCGAAACCTTAACACCACCTT
TTTTGACCCCCGCAGGAGGAGG

EP_T4_3 *Micropterus cataracta*e
GAGCCGGAATAGTGGGCACAGCCCTAAGCCTGCTAATTCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTGGGAGATGACCAAATCTACAATGTAATTGTAACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATACCCATTATAATTGGAGG
CTTTGGCAACTGACTTGTCCCCCTAATGATCGGTGCCCCCGACATAGCATTCC
CCCGAATAAATAACATAAGCTTTTGACTTCTTCCCCCCTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAAGCCGGAGCTGGCACTGGGTGAACTGTTTACCC
CCCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCT
TCTCTCTTCATCTTGCTGGTGTCTCCTCCATCCTAGGGGCCATCAATTTTATTA
CCACAATCATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCT
CTTTGTCTGATCCGTTCTGATTACTGCTGTCCTGCTCCTTCTATCACTCCCAGT
CCTCGCTGCAGGCATTACGATGCTCTTAACAGACCGAAACCTTAACACCACC
TTTTTTGACCCCCGCAGGAGGAGG

***Micropterus coosae* (n = 18)**

DB_T3_1 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTGATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATCATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTGGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCAGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

DB_T2_3 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTAATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATCATAATTGGAGG

CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTAGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCAGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

DB_T2_5 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTGATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATCATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTAGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCGGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCGGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

DB2_T5_1 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTGATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATCATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTAGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCGGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCGGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

CM_T4_5 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTGATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATCATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT

CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTGGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCAGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

CM_T4_4 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTGATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATCATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTAGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCGGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCGGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

DB_T2_4 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTGATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATCATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTGGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCAGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

DB_T2_12 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTGATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATCATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTGGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC

TCGCCGCAGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

DB_T2_2 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTGATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATCATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTGGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCAGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

DB_T2_13 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTGATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCTATCATAATCGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTAGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCAGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

CM2_T6_3 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTGATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATCATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTGGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCAGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

DB_T2_14 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTGATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATCATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTGGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCAGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

DB_T2_1 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTAATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATCATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTAGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCAGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

DB_T4_6_1 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTGATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCTATCATAATCGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTAGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCAGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

DB_T3_3 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTGATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCTATCATAATCGGAGG

CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTAGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCAGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

EP_T1_9 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTGATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATCATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTGGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCAGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

DB_T13_7_COI-F *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTAATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATCATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT
CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTAGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCAGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG

DB_T6_7 *Micropterus coosae*
GAGCCGGAATAGTGGGCACAGCCCTGAGCCTGCTGATCCGTGCAGAACTTAG
CCAACCGGGCGCTCTTCTAGGAGATGACCAAATCTACAATGTAATTGTTACA
GCACATGCATTTGTAATAATTTTCTTTATAGTAATGCCCATCATAATTGGAGG
CTTTGGCAACTGACTTATCCCCCTAATGATCGGTGCCCCCGACATGGCGTTCC
CTCGAATAAACAACATAAGCTTTTGACTCCTTCCCCCTTCCTTCCTTCTCCTGC
TCGCCTCTTCCGGGGTCGAGGCCGGAGCTGGCACTGGGTGGACTGTTTATCCC
CCTCTTGCCGGCAACCTAGCCCATGCAGGAGCATCCGTTGACCTAACCATCTT

CTCTCTTCATCTCGCAGGTGTCTCTTCTATCCTGGGCGCCATCAATTTTATTAC
CACAATCATTAATATAAAACCCCCAGCCATCTCCCAGTACCAAACACCCCTCT
TTGTCTGATCCGTCCTAATTACTGCCGTCCTGCTCCTTCTATCACTCCCAGTCC
TCGCCGCAGGCATTACGATGCTCCTTACGGACCGAAACCTTAACACCACCTTC
TTTGACCCCGCAGGAGGAGG