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Molecular and Morphological Analyses of the Black Basses: Implications for Taxonomy and Conservation

TODD W. KASSLER
Illinois Natural History Survey, Center for Aquatic Ecology
607 E. Peabody, Champaign, Illinois 61820, USA

JEFFREY B. KOPPELMAN
Missouri Department of Conservation, Fish and Wildlife Research Center
1110 S. College Avenue, Columbia, Missouri 65201, USA

THOMAS J. NEAR
University of California-Davis, Center for Population Biology
One Shields Avenue, Davis, California 95616

CASEY B. DILLMAN
Division of Biological Sciences, University of Missouri
105 Tucker Hall, Columbia, Missouri 65211, USA

JEFFREY M. LEVENGOOD
Illinois Natural History Survey, Center for Wildlife Ecology
607 E. Peabody, Champaign, Illinois 61820, USA

DAVID L. SWOFFORD
Florida State University, School of Computational Science and Information Technology
and Department of Biological Science, Tallahassee, Florida 32306, USA

JEFFREY L. VANORMAN, JULIE E. CLAUSSEN, AND DAVID P. PHILIPP
Illinois Natural History Survey, Center for Aquatic Ecology
607 E. Peabody, Champaign, Illinois 61820, USA

Abstract.—Taxonomists currently recognize seven species and three subspecies in the genus *Micropterus*. Based on variation in meristic characters, allozymes, and mtDNA, two subspecies are clearly distinct from one another and warrant elevation to species status. *Micropterus salmoides floridanus* should now be recognized as the Florida bass *M. floridanus*, and *M. salmoides salmoides* as the largemouth bass *M. salmoides*. Although the Alabama spotted bass *M. punctulatus henshalli* is morphologically and genetically quite distinct from the northern spotted bass, a thorough taxonomic assessment is still required prior to any revision. The status of a third subspecies, Neosho smallmouth bass *M. dolomieu velox*, was not investigated. Phylogenetic analyses of mitochondrial DNA sequence variation indicate that the genus is represented by four lineages: (1) smallmouth bass *M. dolomieu* and spotted bass *M. punctulatus*; (2) largemouth bass *M. salmoides*, Florida bass *M. floridanus*, Suwannee bass *M. notius*, and Guadalupe bass *M. treculi*; (3) shoal bass *M. cataractae*; and (4) redeye bass *M. coosae* and Alabama spotted bass. It is likely that through either natural or human-induced changes, hybridization has occurred between the Alabama spotted bass and *M. coosae* and between *M. punctulatus* and *M. treculi*, which may have obscured the true phylogenetic affinities of these taxa. In response to this new information, management agencies need to alter their policy toward stocking non-native species and promoting stock transfers. Specifically, they should terminate Florida bass stocking programs outside of Florida.

Introduction

Although seven species and three subspecies are currently recognized in the genus *Micropterus*, this group has undergone many changes in classification over the years. Lacépède first described the genus *Micropterus* in 1802, and subsequently, six synonymous groups were added to this genus by early taxonomists (Hubbs and Bailey 1940). Henshall (1881) provided a full synonymy for *Micropterus*, including both the original characterizations and those provided by later taxonomists. Collectively, early taxonomists used 8 generic and 20 specific names to identify the various taxa in *Micropterus* (Ramsey 1975). Jordan and Evermann (1896) consolidated all of the previous classifications into just two species, largemouth bass *M. salmoides* and smallmouth bass *M. dolomieui*. Hubbs (1926) applied the genus name *Aplites* to the largemouth bass and, one year later (Hubbs 1927), described a third species (spotted bass *M. pseudaplites*) that was later considered to be a synonym of *M. punctulatus* (Rafinesque 1819). Hubbs and Bailey (1940) changed the largemouth bass genus from *Aplites* to *Huro* and recognized all other black bass taxa as *Micropterus*. In their revision, Hubbs and Bailey (1940) recognized one new species (redeye bass *M. coosae*) and three new subspecies (Wichita spotted bass *M. p. wichitae*, Alabama spotted bass *M. p. henshalli*, and Neosho smallmouth bass *M. d. velox*), in addition to the northern smallmouth bass *M. d. dolomieui*, northern spotted bass *M. p. punctulatus*, and largemouth bass *Huro salmoides*. De Buen (1941) questioned the status of the genus *Huro* because of the close resemblance of the species in the two genera *Huro* and *Micropterus*. As a result, the monotypic *Huro* was subsumed into *Micropterus* (Bailey and Hubbs 1949). Bailey and Hubbs (1949) also described the Florida largemouth bass *M. s. floridanus* as a new subspecies with northern largemouth bass *M. s. salmoides*.

Several currently recognized species of black bass were initially considered subspecies. Guadalupe bass *M. treculi* was described originally as a form of *M. salmoides* from Texas (Baird and Girard 1854). Hubbs and Bailey (1940) later synonymized *M. treculi* with *M. p. punctulatus*. Upon re-examination, Hubbs and Bailey (1942) reclassified *M. p. treculi* as a subspecies of *M. punctulatus*. Upon the discovery of sympatry with *M. p. punctulatus* in the San Marcos and Guadalupe Rivers (Hubbs 1954), *M. treculi* was finally classified as a distinct species. In addition, *M. p. wichitae* was described as a subspecies of *M. punctulatus* (Hubbs and Bailey 1940) but was later invalidated through assessment of stocking records (Cofer 1995). The shoal bass *M. cataractae* was originally recognized as a form of *M. coosae* (Ramsey 1975) and has only recently been described as a distinct species (Williams and Burgess 1999).

Hybridization between several taxa of *Micropterus* has been documented: *M. s. floridanus* and *M. s. salmoides* (Philipp et al. 1981, 1983a, 1983b, 1985a, 1985b; Wright and Wigtil 1981; Parker et al. 1985; Williamson et al. 1986; Fields et al. 1987; Koppelman et al. 1988; Philipp 1991, 1992; Philipp and Whitt 1991; Nedbal and Philipp 1994; Gelwick et al. 1995; Alvarado Bremer et al. 1998); *M. treculi* and *M. dolomieu* (Edwards 1979; Whitmore and Butler 1982; Whitmore 1983; Morizot et al. 1991); *M. dolomieu* and *M. p. punctulatus* (Pflieger and Fajen 1975; Koppelman 1994; Pierce and Van Den Avyle 1997); *M. dolomieu* and *M. coosae* (Turner et al. 1991; Pipas and Bulow 1998) *M. s. salmoides* and *M. dolomieu* (Wheat et al. 1971; Whitt et al. 1971; Beaty and Childers 1980; Buck and Hooe 1986; Whitmore and Hellier 1988); *M. coosae* and *M. cataractae* (Dunham et al. 1994).

Although several species of *Micropterus* have been intensively studied from an ecological and fisheries management standpoint, few hypotheses exist concerning the phylogenetic relationships among the species and subspecies in the genus. In addition, although population level genetic data exist for some of the taxa, conclusive molecular analyses investigating the phylogenetic relationships among the various taxa in the genus have not been completed. Morphological characteristics traditionally have been used in taxonomy and systematics, but molecular genetic data are commonly accepted in studies of congeneric relationships. The goal of this study was to assess the genetic relationships among the various taxa currently recognized in *Micropterus*. Our approach was to sample individuals from a few representative populations across the range of each taxon and to assess their relationships using four different techniques: an analysis of morphological variation using meristic data; an analysis of variation in the nuclear genome using allozymes; and two analyses of variation in mtDNA, RFLP analysis of the ND-3/4 mitochondrial genes and sequence analysis of the ND-2 and Cytochrome b (cyt b) genes. Recommendations on the taxonomy of the taxa in this group are made based upon these assessments. Phylogenetic relationships among these taxa, however, are based primarily on the mtDNA sequence analyses. In keeping with standard practice, due to the taxo-

nomic nature of this paper, binomial nomenclature will be used throughout; common names are given when informative.

Material and Methods

Collections

Sample localities for nine taxa were selected to provide representative samples across the geographical range of each taxa (Table 1). Due to species elevation recommendation for *M. s. floridanus*, samples representing that taxon will be referred to as *M. floridanus* (common name, Florida bass) throughout the remainder of this paper. In response, *M. s. salmoides* will be treated as *M. salmoides* (common name, largemouth bass). Because it also became apparent during the analyses that presumed samples of *M. d. velox* from the Elk River, Missouri, were the same as *M. d. dolomieu*, all samples representing that species are referred to as *M. dolomieu* (common name, smallmouth bass). Finally, all of the samples of Alabama spotted bass were collected from Jordan Lake, Alabama. The true taxonomic status of this group is called into question as a result of our findings; however, assignment of a new binomial classification awaits further investigation. Consequently, throughout this paper, we refer to this group by its current common name, Alabama spotted bass.

Fish were collected using a variety of sampling techniques, including electrofishing, seining, and angling. Most samples were whole fish that were frozen at –20°C until shipped to the Illinois Natural History Survey in Champaign, Illinois (INHS), for analysis. Additional samples were fin clips stored in 95% ethanol prior to being shipped to the INHS for analysis.

Morphological Analysis

A total of 20 meristic characters were enumerated, as described by Hubbs and Lagler (1958), for whole fish samples. Counts for each individual were analyzed using a stepwise discriminant function analysis. The analysis was performed using the DISCRIMINANT protocol of SPSS (1998) with F-to-enter set at 0.05 and F-to-remove at 0.10. Stepping was accomplished using the WILKS specification of the METHOD subcommand. Under this procedure, the variable that minimizes Wilk's lambda is entered. Prior probabilities were set equal to sample sizes to account for disparity in group size. Polygons representing species data points were produced pairwise for the most discriminatory functions.

Protein Electrophoretic Analysis

Samples of white skeletal muscle, eye, and liver were dissected from whole frozen fish, thawed, homogenized in 100 mM Tris-HCl (pH 7.0), and centrifuged at 22,000 × g for 10 minutes at 4°C. Tissue extracts were subjected to vertical starch gel electrophoresis coupled with histochemical staining, as described by Philipp et al. (1979), with modifications from Koppelman and Philipp (1986).

Because the most common allele for each locus varied among species, the relative mobility of each allele for all samples was determined following assignment of a value of 100 for the least anodally migrating allele detected among all the species. Allele frequencies, percent polymorphic loci, and mean heterozygosities were calculated using BIOSYS-1 (Swofford and Selander 1981). Allozyme data were analyzed phylogenetically by the frequency parsimony method (Swofford and Berlocher 1987), using the MANOB approximation method described by Berlocher and Swofford (1997), as implemented in PAUP* (Swofford 2002).

DNA Isolation

Genomic DNA (for use in both RFLP analysis and sequence analysis of mtDNA) was isolated using a technique described by Saghai-Maroof et al. (1984) and modified by Fields et al. (1989). Approximately 200 mg of white skeletal muscle was ground gently in a solution of 100 mM Tris-HCl (pH 8.0), 10 mM EDTA, 1.4 M NaCl, 2% CTAB (Hexadecyltrimethyl-ammonium bromide), and 0.2% 2-mercaptoethanol. Proteinase K was added to a final concentration of 10 µg/mL, and the sample was then incubated at 60°C for 35 min. One phenol extraction and two chloroform:isoamyl alcohol (24:1) extractions were used to remove cellular debris and denatured protein from the DNA. DNA was precipitated overnight at –20°C in 95% ethanol, then centrifuged at 12,000xg, air-dried, and resuspended in ddH_2O.

RFLP Analysis of mtDNA

RFLP analysis was performed on a PCR-amplified segment (ND-3/4) of the mitochondrial genome that included two genes (NADH subunits 3 and 4). Approximately 2,200 base pairs from this region were amplified using a light strand primer (L9386) that is located in the $tRNA^{Gly}$ gene (5'-GTACACGTCACTTCCAATCA-3'; Park et al., 1993) and a heavy strand primer (H11540) located in the $tRNA^{His}$ gene (5'-AGAATCACAATCTAATGTTT-3'; Park et al. 1993). The polymerase chain reaction mixture modified from Echt et al. (1991) contained the fol-

Table 1. Populations and number of individuals analyzed for each technique: meristic analysis of 20 characters; allozyme analysis of 19 polymorphic loci; restriction fragment length polymorphism (RFLP) analysis of the ND-3/4 mitochondrial regions with four restriction endonucleases, and sequence analysis of 1,047 base pairs in the ND-2 gene and 1,140 base pairs in the cytochrome-b gene within the mitochondrial genome.

				Number of individuals analyzed for each technique			
#	Species	Location	County	Meristics	Allozymes	RFLPs	Sequences
1	*M. salmoides*	Lake Shelbyville, IL	Moultrie	4	20	20	0
2	*M. salmoides*	Lipsett Lake, WI	Burnett	0	30	19	1
3	*M. salmoides*	Howard Lake MN	Wright	10	20	0	0
4	*M. salmoides*	Waneta-Lamoka, NY	Schuyler	7	20	0	0
5	*M. salmoides*	Fish Lake, MI	Delta	0	20	0	0
6	*M. salmoides*	Long Lake, Ont	Frontenac	0	0	0	1
7	*M. salmoides*	Nueces River, TX	Uvalde	0	0	0	2
8	*M. floridanus*	Lake Eustis, FL	Lake	5	30	20	1
9	*M. floridanus*	Lake Istokpoga, FL	Highlands	8	20	6	1
10	*M. floridanus*	Lake Okeechobee, FL	Glades	8	20	0	0
11	*M. floridanus*	Lake George, FL	Marion	0	20	0	0
12	*M. floridanus*	Hillsborough River, FL	Hillsborough	7	20	0	0
13	*M. dolomieu*	James River, VA	Henricho	0	30	21	1
14	*M. dolomieu*	Fox River, WI	Kenosha	13	30	20	0
15	*M. dolomieu*	Lake St. Clair, MI	St. Clair	14	20	0	0
16	*M. dolomieu*	Lake Cumberland, KY	Russel	0	20	0	0
17	*M. dolomieu*	Lake Winnebago, WI	Winnebago	0	30	0	0
18	*M. dolomieu*	Miller's Lake, Ont	Frontenac	0	0	0	1
19	*M. dolomieu*	Elk River, MO	McDonald	0	20	37	4
20	AL spotted bass	Jordan Lake, AL	Elmore	23	20	10	2
21	*M. punctulatus*	Norris Reservoir, TN	Union	21	20	20	0
22	*M. punctulatus*	Chase Lake, KS	Chase	0	30	18	2
23	*M. punctulatus*	Lake Texoma, OK	Marshall	0	40	0	0
24	*M. punctulatus*	Lake Cumberland, KY	Russel	0	17	0	0
25	*M. punctulatus*	Ross Barnett Reservoir, MS	Rankin	34	19	0	0
26	*M. coosae*	Shoal Creek, AL	St. Clair	20	20	0	0
27	*M. coosae*	Black Warrior River, AL	Hale	20	18	0	0
28	*M. coosae*	Coosa River, AL	Cherokee	19	20	15	3
29	*M. coosae*	Tallapoosa River, AL	Chambers	19	20	15	2
30	*M. coosae*	Snake Creek, GA	Carroll	0	20	0	0
31	*M. coosae*	Conasauga River, TN	Polk	0	0	0	1
32	*M. cataractae*	Flint River, GA	Crisp	12	13	10	1
33	*M. cataractae*	Little Uchee Creek, AL	Russel	0	12	10	1
34	*M. cataractae*	Chatahoochie River, GA	Early	12	20	0	0
35	*M. notius*	Suwannee River, FL	Suwannee	17	20	0	1
36	*M. notius*	Sante Fe River, FL	Alachua	16	20	13	0
37	*M. notius*	Wacissa River, FL	Jefferson	0	21	13	1
38	*M. treculi*	Guadalupe River, TX	Kerr	40	7	9	0
39	*M. treculi*	Johnson Creek, TX	Kerr	0	20	20	2
40	*M. treculi*	Nueces River TX	Uvalde	0	20	22	0
41	*M. treculi*	Llano River, TX	Kimble	0	0	0	2

lowing: approximately 25 ng of template DNA, Applied Biosystems 10X Buffer, 2.5 mM $MgCl_2$, 100 µM each of dATP, dCTP, dGTP, and dTTP, 0.08 pmol/µL each of the light and heavy strand primers, and 1.5 units *Taq* polymerase for every 50 µL reaction. Amplification was performed using an MJ Research PTC-100-60 thermocycler with the following profile: an initial denaturation step of 2 min at 95°C, followed by 45 sec at 94°C, 1 min at 45°C, and 2 min 30 sec plus a 4 sec extension/cycle at 70°C. This protocol was repeated for 29 cycles, followed by a final extension step at 70°C for 4 min, plus a final holding step at 4°C.

Subsamples (8µL) of each amplified PCR product were digested with each of four restriction endonucleases (*Dpn II*, *Hha I*, *Msp I*, and *Rsa* I), following manufacturer's recommendations. Digests were separated electrophoretically in a gel consisting of 2.0% agarose and 1.0% Synergel (Diversified Biotech) in TAE buffer (0.04 M Tris-HCl, 5.7%

glacial acetic acid, and 0.001 M EDTA, pH 8.0) with 0.5 µg/mL ethidium bromide to visualize banding patterns under ultraviolet light. Black and white Polaroid photographs were taken of each gel to use in measuring size of fragments for each banding pattern. A 100 base pair DNA ladder (0.5 µg/lane) was used as a standard for fragment-size determination.

Composite haplotypes were constructed by assigning a letter to each banding pattern observed for each restriction enzyme digest. Each composite haplotype consisted of four letters that corresponded to each of the four enzymes.

Sequence Analysis of mtDNA

PCR amplification of the 1,140 base pair cyt b and 1,047 base pair ND-2 genes were each performed in a 50 µL reaction volume with 1 µL of total genomic DNA. The two primers used for the amplification of the cyt b gene were HA: (5′ CAACGATCTCCGGTTTACAAGAC) and LA: (5′ GTGACTTGAAAAACCACCGTTG), and the primers used to amplify the ND-2 gene were previously published (Kocher et al. 1995). The concentrations of remaining reaction components were 1x Taq buffer, 0.4 mM each primer, 0.2mM each dNTP, 2 mM $MgCl_2$, 1 unit (0.2mL) of Taq DNA polymerase (Life Techologies, Inc.), 10% DMSO (v/v), and ddH_2O to final volume. The thermal cycling profile for amplification of both genes was an initial denaturation step of 94°C for 4 minutes, followed by 35 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 1 minute with a final extension of 4 minutes at 72 °C. After amplification, 3–6 µL of the PCR product was screened on 1.0% agarose gels to ensure that the proper fragment had been amplified.

The cyt b PCR products were cloned for sequencing. A 6 µL aliquot of the PCR product was ligated (4°C for 16 hours) into the pGEM T-Easy vector (Promega Corp.), as directed by the manufacturer. A 25 mL sample of competent E. coli (JM109; Promega Corp.) was transformed with 4 mL of the ligation reaction, as directed by the manufacturer. Transformed cells were grown overnight at 37°C on LB/ampicillin plates with IPTG/X-gal. The colonies were screened for positive transformation by standard blue/white colony screening, as provided for by the pGEM T-easy kit. Colonies that were positive for transformation (white colonies) were selected and grown for 18 hours in LB broth at 37°C. Plasmid mini-preps were completed with the QIAGEN plasmid mini-kit, as directed, and resuspended into 50 mL of ddH_2O.

Both plasmids and PCR products (ND-2 PCR was sequenced directly) were sequenced using BigDye Terminator cycle sequencing. For cyt b sequences, the first set of reactions used the plasmid primers M13 forward (5′-CGTTGTAAAACGACGGCGAGT) and M13 reverse (5′-CAGGAAACACGTATGACCATG). The primers HA 1 (5′-ACGATRGGHACRACYATD) and LA1 (5′-GCAACCGCCTTCTCATC) were designed to sequence the internal portion of cyt b. Two internal primers were used to sequence both strands of ND-2, bassnd2-f1 (TRAACCAAACCCARCTCCGRAAAAT) and bassnd2-r1 (ATTGTAAGATGAGRATTATTC). Sequencing reaction mixtures were then analyzed on either an ABI 3700 or an ABI 310 sequencer.

Cyt b and ND-2 sequences were concatenated into a single data file along with existing sequences for the centrarchid outgroup taxa *Ambloplites rupestris* (from Lake Andrusia, Beltrami County, Minnesota) and *Lepomis punctatus* (from the Conasauga River, Bradley County, Tennessee). A partition homogeneity test (Farris et al. 1994) was used to evaluate the null hypothesis that the two genes represent a random division of the entire pool of nucleotide sites into two subsets. Failure to reject this null hypothesis provides some reassurance that combining data from the two genes for further analysis is reasonable. Subsequent phylogenetic analyses were performed under maximum-likelihood and parsimony criteria using version 4.0b10 of PAUP* (Swofford 2002). Model selection for likelihood analyses was initiated from a neighbor-joining tree (Saitou and Nei 1987) using the LogDet distance transformation (Lockhart et al. 1994). A model was chosen by starting from the most complex model implemented in PAUP* (the general time-reversible model with gamma-distributed rates and an unknown proportion of invariable sites) and searching for simpler models that provided adequate fit to the observed data but required estimation of fewer parameters.

For both parsimony and likelihood analyses, heuristic searches for optimal tree topologies were conducted using 100 random-addition-sequence starting points followed by TBR branch swapping (Swofford et al. 1996). For likelihood analysis, a successive approximations approach to parameter estimation was used, as outlined in Swofford et al. (1996). Model parameters were optimized on the initial neighbor-joining tree and then fixed to these estimates for the first tree search. The parameters were then re-estimated on the tree found by this search, and the process was repeated until the same tree was found in two consecutive tree searches.

Parsimony analyses were conducted using equally weighted characters, as well as a variety of weighting schemes that assigned greater weight to first and second codon positions and/or greater cost to transversions than transitions. Confidence in phylogenetic groupings (nodal support) was assessed using nonparametric bootstrapping (Felsenstein 1985) with 100 and 1,000 replicates for likelihood and parsimony, respectively.

Results

Morphological Analysis

Stepwise discriminant function analysis of the 20 meristic characters was counted as predictors of group membership. After stepping, 14 characters remained in the analysis (Table 2). A summary of mean values and ranges of the 14 meristic characters used for each taxon is provided in Appendix 1. A total of eight functions were calculated; combined, these functions contained significant discriminating power ($X^2_{(112)} = 1{,}687$; $P<0.001$). The first, second, and third functions accounted for 38%, 30%, and 18% of the between-group variability, respectively. The loading matrix of correlations between predictor variables and the first three functions revealed that different combinations of meristic characters contributed most to group discrimination (Table 2). The number of scales above the lateral line, on the lateral line, caudal peduncle, and opercle, and the number of dorsal and pectoral rays were the primary distinguishing variables on the first three functions.

Plots of discriminant scores based on pairwise combinations of the first three functions alone revealed that the different taxa were not completely distinguished on the combined axes (Figure 1). Each pairwise function plot had considerable overlap of taxa; use of all functions was required to classify the individuals in each taxon at the reliability shown in Table 3. For example, in the plot of Function 1 versus Function 2 (Figure 1 A), *M. floridanus* was distinguished substantially from all other groups, although there still was a 9 percent overlap in the polygons of *M. floridanus* and *M. salmoides*. The plot of Function 2 versus Function 3 (Figure 1B) shows a much greater overlap of *M. floridanus* with *M. salmoides*, demonstrating the need to use multiple functions for best distinguishing among taxa. When all eight functions were used, 86 percent of the specimens were classified correctly. Individual classification rates were reasonably high for all species except *M. coosae*, which overlapped greatly (15%) with *M. treculi* and to a lesser degree with *M. salmoides*, *M. punctulatus*, *M. cataractae*, and Alabama spotted bass (Table 3).

Protein Electrophoretic Analysis

Of the 29 loci visualized, 19 were polymorphic among the nine taxa (Table 4; Appendix 2). Fixed differences were observed among all taxa except between *M. punctulatus* and *M. treculi* (Table 4). Variability estimates (Table 5) based on all individuals representing each species were lowest for *M. notius* (percent polymorphic loci P = 3.4%) and *M. punctulatus* and *M. treculi* (observed heterozygosity H = 0.006) and highest for *M. coosae* (P = 17.2%; H = 0.045).

Frequency parsimony analysis for the 36 populations produced 612 equally parsimonious trees.

Table 2. Meristic characters used to discriminate among *Micropterus* spp. Functions one, two, and three accounted for 86 percent of the among-group variability.

Variable	Function 1	Function 2	Function 3
scales above lateral line	0.56	−0.34	−0.09
scales on lateral line	0.32	−0.04	−0.30
scales on caudal peduncle	0.21	0.62	0.36
scales below lateral line	0.20	−0.12	0.28
scales on cheek	0.15	−0.26	−0.16
scales before dorsal fin	0.28	−0.27	0.11
dorsal rays	0.35	0.22	−0.40
scales on interopercle	0.19	0.05	0.04
scales on opercle	−0.15	−0.80	0.16
gill rakers	0.28	0.13	−0.20
anal rays	0.06	0.27	−0.18
scales on subopercle	0.15	0.42	0.09
pectoral rays	0.05	−0.15	0.34
spinous sepum	0.07	−0.05	0.57

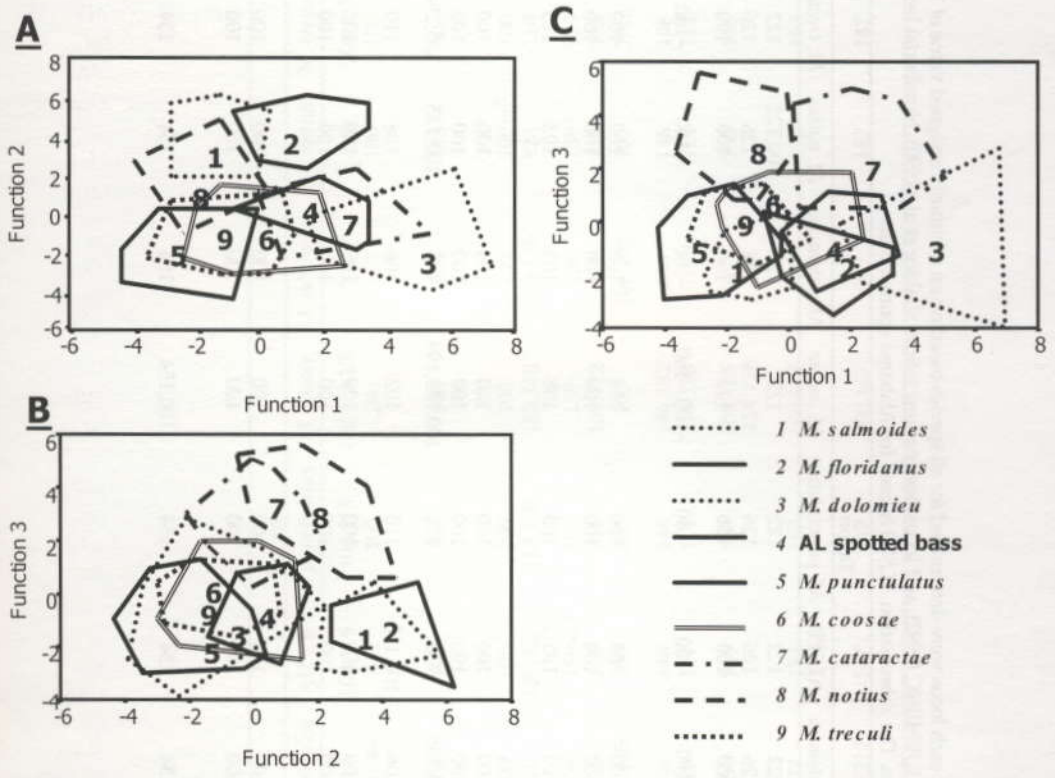

Figure 1. Discriminant function analysis of meristic characters for the nine taxa within *Micropterus*. Panel A is function 1 vs. function 2; panel B is function 2 vs. function 3; panel C is function 1 vs. function 3.

These trees differed only in trivial rearrangements involving conspecific populations; one example is shown in Figure 2. Conspecific populations generally group together, with a notable exception involving intermingling and low differentiation between *M. treculi* and *M. punctulatus* populations. The allozyme data do not support the distinctiveness of the *M. dolomieu* from the Elk River (presumed Neosho subspecies). The allozyme data and tree do, however, support the genetic distinctive-

Table 3. The accuracy of meristic characters for identification of *Micropterus* spp. Classification is based on discriminant function, using three functions that accounted for 86 percent of the variability observed. Misclassifications are presented as the proportion assigned incorrectly to the given taxon.

Taxa	N	Percent correct classification	Misclassifications
M. salmoides	21	100	
M. floridanus	24	91.7	8.3% *M. salmoides*
M. dolomieu	29	96.6	3.4% AL spotted bass
AL spotted bass	23	100	
M. punctulatus	54	90.7	5.6% *M. coosae*, 3.7% *M. treculi*
M. coosae	73	69.9	15.1% *M. treculi*, 5.5% *M. punctulatus*, 5.5% *M. cataractae*, 2.7% AL spotted bass, 1.4% *M. salmoides*
M. cataractae	24	91.6	4.2% *M. coosae*, 4.2% *M. notius*
M. notius	30	100	
M. treculi	35	85.7	11.4% *M. coosae*, 2.9% *M. punctulatus*

Table 4. Alleles present among the taxa of black bass. Relative allelic mobilities (Rf) at each locus were determined for all species based on an arbitrarily assigned value of 100 to the least migrating allele observed. Enzyme numbers are those recommended by IUBMBNC (1992), and locus designations follow Shaklee et al. (1990). Number(s) listed under each taxon corresponds to Rf for the allele(s) at each locus present in that taxon. The abbreviation ALSPB is used for Alabama spotted bass.

Enyme number	Enzyme name	Locus	Taxon								
			M. salmoides	*M. floridanus*	*M. dolomieu*	ALSPB	*M. punctulatus*	*M. coosae*	*M. cataractae*	*M. notius*	*M. treculi*
2.6.1.1	Aspartate amino transferase	*sAAT-A**	122	122,130	122	122	122	122	122	100,122	122
		*sAAT-B**	100,110	126,139	126	126	126	126,139	126	126	126
		*mAAT**	100	100	100	100	100	100	100	100	100
1.1.1.1	Alcohol dehydrogenase	*ADH**	−100	−100	−100	−100	−100	−100,−160	−100	−100	−100
2.7.4.3	Adenlyate kinase	*AK-1**	100	100	100	100	100	100	100	100	100
		*AK-2**	100	100	100	100	100	100	100	100	100
———	Calcium binding protein	*CBP**	103	103	100	105	103	108	103	103	103
2.7.3.2	Creatine kinase	*CK-A**	100	100	100	100	100	100	100	100	100
		*CK-B**	100	100,105	100	100	100	100	100	100	100
		*CK-C**	100,135	100,135	100,135	135	135	100,135,194	135	100,135	135,194
3.1.1.-	Esterase	*EST**	106	106	106	100,110	110	102	106	106	110
4.1.2.13	Fructose 1,6-bisphosphate aldolase	*FBALD-1**	100	100	100	100	100	100	100	100	100
		*FBALD-2**	100	100	100	100	100	100	100	100	100
1.2.1.12	Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH-1**	100	100	100	100	100	100	100	100	100
		*GAPDH-2**	100	100	100	100	100	100	100	100	100
1.1.1.8	Glycerol-3-phosphate dehydrogenase	*G3PDH**	154	154	154	154	154	100,154	154	154	154

Table 4. Continued.

Enyme number	Enzyme name	Locus	Taxon								
			M. salmoides	*M. floridanus*	*M. dolomieu*	ALSPB	*M. punctulatus*	*M. coosae*	*M. cataractae*	*M. notius*	*M. treculi*
5.3.1.9	Glucose phosphate isomerase	*GPI-A**	115	100,115	115	100,115	100,115	100,115,130	115	115	100,115
		*GPI-B**	100,144	100,144	100,144	100	100	100	100	100	100
1.1.1.14	L-Idditol dehydrogenase	*IDDH**	100	100	100	100	100	100	100	100	100
1.1.1.42	Isocitrate dehydrogenase	*sIDHP-A**	100	100	100	100	100	100	100	100	100
		*sIDHP-B**	100	120	120	100,120	110,130	120,140	120,140	120	110
1.1.1.27	Lactate dehydrogenase	*LDH-A**	159	100,159	159	159	159	159	159	159	159
		*LDH-B**	122	122	122	122	122	100,122	122	122	122
		*LDH-C**	105	102,105	100,105	105	105	105	100,105	105	105
1.1.1.37	Malate dehydrogenase	*sMDH-A**	146	100,146	146	146	146	146,182	146	146	146
		*sMDH-B**	100,114	114	128	114	114	114,128	114	114	114
1.1.1.43	Phosphogluconate dehydrogenase	*PGDH**	100	100	100	100	100	100,108	100	100	100
2.7.5.1	Phospho-glucomutase	*PGM**	153,175	153	137	153	153	100,137	153	153	153
1.15.1.1	Superoxide dismutase	*SOD**	147	100,147	147,212	147	147	100,147	147	147	147

Table 5. Summary of allozyme variation detected among the taxa of black bass. Sample size, percentage of loci polymorphic, and heterozygosity (H; observed and Hardy-Weinberg expected with standard errors in parenthesis) are shown for each taxon.

Species	Percentage of N	Mean heterozygosity loci polymorphic	Observed	H-W expected
M. salmoides	110	6.9	0.016 (0.009)	0.023 (0.013)
M. floridanus	110	10.3	0.036 (0.020)	0.041 (0.023)
M. dolomieu	150	6.9	0.021 (0.014)	0.030 (0.020)
AL spotted bass	20	10.3	0.019 (0.011)	0.020 (0.012)
M. punctulatus	126	6.9	0.006 (0.004)	0.010 (0.007)
M. coosae	98	17.2	0.045 (0.018)	0.052 (0.020)
M. cataractae	45	6.9	0.010 (0.007)	0.011 (0.008)
M. notius	61	3.4	0.008 (0.007)	0.009 (0.008)
M. treculi	47	6.9	0.006 (0.004)	0.008 (0.006)

ness of *M. floridanus* relative to *M. salmoides*, as well as a strong separation of the Alabama spotted bass from other *M. punctulatus* populations.

RFLP Analysis of mtDNA

Four restriction endonucleases (*Dpn II*, *Hha I*, *Msp I*, and *Rsa I*) cleaved at 34 sites within the ND-3/4 mitochondrial gene amplification product, producing 14 composite haplotypes (Table 6). *Micropterus salmoides* was the only taxon fixed for a single, unique haplotype (AAAA); *M. floridanus* had two unique haplotypes (GABA and GACA). Two species, *M. coosae* and *M. cataractae*, had three different haplotypes each, although the composites differed by only one or two restriction enzymes for each species. One of the three *M. coosae* haplotypes was shared with all of the Alabama spotted bass individuals that were examined. *Micropterus dolomieu* had only a single haplotype (BBDB) that was shared with *M. punctulatus* and *M. treculi*. *Micropterus treculi* had two haplotypes, the one shared with *M. dolomieu* and *M. punctulatus*, and CCEF. All three collection localities for *M. treculi* had both types.

Sequence Analysis of mtDNA

Sequence information is accessible on Genbank. Sequence divergence between species ranged from 1.02 percent between *M. punctulatus* and *M. dolomieu* to 10.48 percent between *M. dolomieu* and *M. notius* (Table 7). Sequence divergence among conspecific individuals was as low as 0.14 percent (*M. punctulatus* and *M. treculi*) and as high as 2.60 percent (*M. coosae*). In fact, individuals of *M. coosae* from the Coosa River drainage were more similar to Alabama spotted bass from the same drainage than to other *M. coosae* from the Tallapoosa River.

In addition, two very distinct mtDNA types were found within *M. treculi*. One of these types is substantially different from the mtDNA of all other species. Extremely low sequence divergence between the second type and mtDNA from *M. punctulatus* raises the possibility of recent hybridization between these two species. Consequently, until more extensive geographic sampling is conducted and additional (nuclear) sequence data are obtained, we provisionally consider the first mtDNA type to represent "true" *M. treculi*.

The partition homogeneity test failed to reject the hypothesis that the cyt b and ND2 genes represent a random partition of the sites ($P > 0.6$). Because of this result, along with the high level of congruence between trees estimated from each gene separately, we report only the analysis of the combined mtDNA data set. To estimate our maximum-likelihood tree, we chose the substitution model of Tamura and Nei (1993) with gamma-distributed rates across sites (Yang 1993). The Tamura-Nei (TN) model assumes three substitution types—purine transitions (changes between nucleotides A and G), pyrimidine transitions (changes between C and T), and transversions (changes between a purine and a pyrimidine)—that may each occur at different rates and for which equilibrium base frequencies are not necessarily equal. The gamma distribution was approximated by four discrete rate categories of equal frequency (Yang 1994). The log likelihood score for the best tree found under this model was –9798.49. Any simpler model fits the data much more poorly. The low frequency of nucleotide G and high frequency of nucleotide C preclude the use of equal-base-frequency models (estimated equilibrium frequencies were 0.24, 0.35, 0.13, and 0.27, for A, C, G, and T, respectively). Additionally, the marked transition bias and strongly different rates between the two transition types cause substitution models simpler than TN to be inadequate (relative purine and pyrimidine

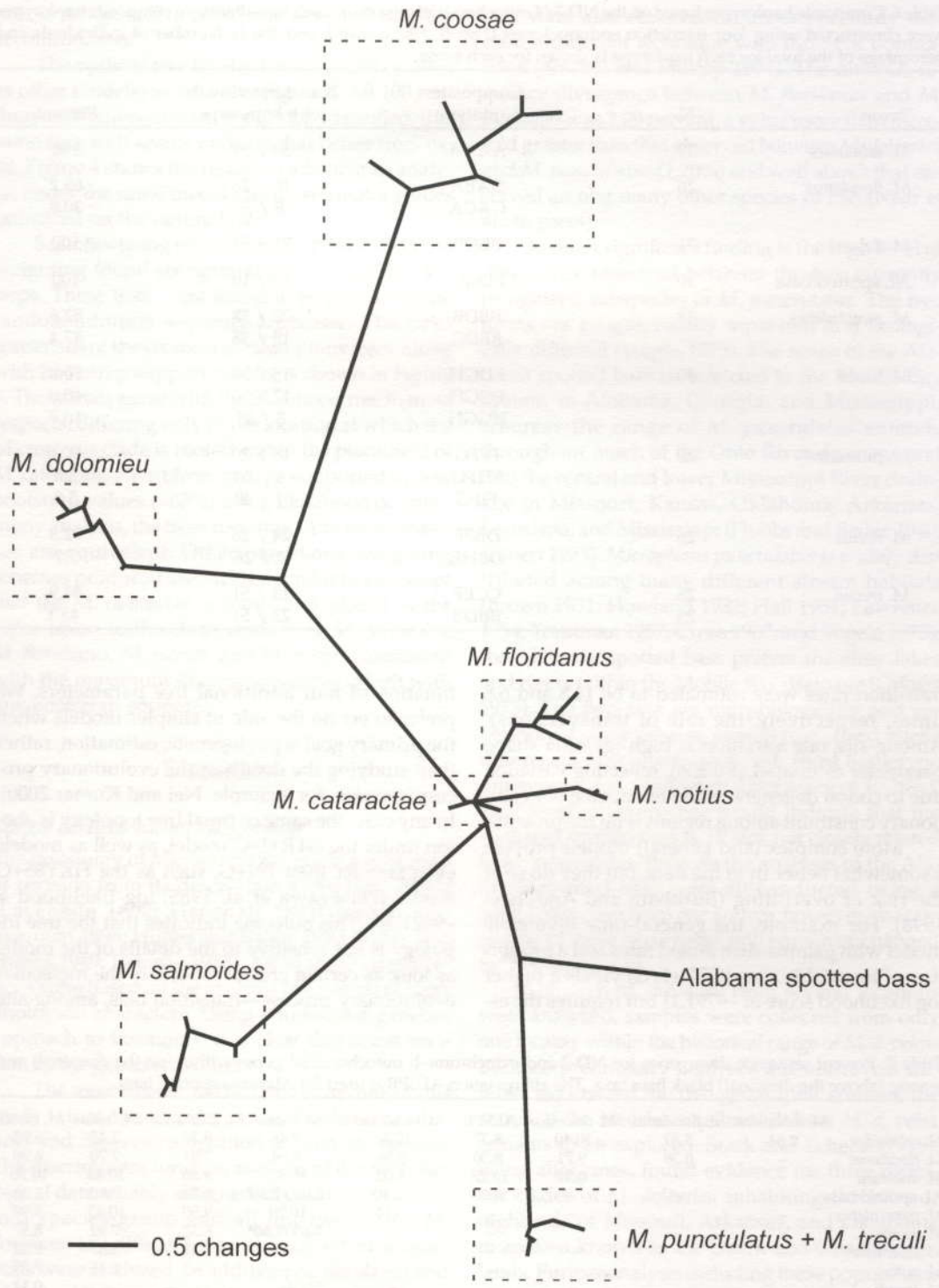

Figure 2. Unrooted tree from frequency parsimony analysis using data from 29 allozyme loci for 36 *Micropterus* populations. Branch lengths are proportional to Manhattan distances between inferred allele frequency arrays. This is one of 612 equally parsimonious trees; other trees differ only in trivial rearrangements involving conspecific populations.

Table 6. Composite haplotypes based on the ND-3/4 mitochondrial regions of black bass. Fourteen composite haplotypes were constructed using four restriction endonucleases (*Dpn II, Hha I, Msp I,* and *Rsa I*). Number of individuals and percentage of the total for each haplotype is shown for each taxon.

Taxon	N	Composite haplotype	No. of individuals with haplotype	Percent
M. salmoides	39	AAAA	39 / 39	100
M. floridanus	26	GABA	18 / 26	69.2
		GACA	8 / 26	30.8
M. dolomieu	71	BBDB	71 / 71	100
AL spotted bass	10	EDGH	10 / 10	100
M. punctulatus	38	BBDB	20 / 38	52.6
		BBDC	18 / 38	47.4
M. coosae	30	EDGH	15 / 30	50.0
		EGGH	12 / 30	40.0
		HGGH	3 / 30	10.0
M. cataractae	20	FFFG	18 / 20	90.0
		FHFG	1 / 20	5.0
		FIFG	1 / 20	5.0
M. notius	26	DBFE	24 / 26	92.3
		DBHE	2 / 26	7.7
M. treculi	28	CCEF	28 / 51	54.9
	23	BBDB	23 / 51	45.1

transition rates were estimated to be 14.8 and 6.8 times, respectively, the rate of transversions). Among-site rate variation is high (gamma shape parameter estimated at 0.236), reflecting variation due to codon degeneracy and the degree of evolutionary constraint among regions with the proteins.

More complex (and general) models provide a somewhat better fit to the data, but they do so at the risk of overfitting (Burnham and Anderson 1998). For example, the general-time reversible model with gamma-distributed rates and a proportion of invariable sites (GTR+I+G) yields a higher log likelihood score of –9791.11 but requires the estimation of four additional free parameters. We prefer to err on the side of simpler models when the primary goal is phylogenetic estimation, rather than studying the details of the evolutionary process (see also, for example, Nei and Kumar 2000). In any case, the same optimal tree topology is chosen under the GTR+I+G model, as well as models even simpler than TN+G, such as the HKY85+G model (Hasegawa et al. 1985; log likelihood = –9821.34). This outcome indicates that the tree topology is not sensitive to the details of the model as long as certain crucial aspects of the molecular evolutionary process—transition bias, among-site

Table 7. Percent sequence divergence for ND-2 and cytochrome-b mitochondrial genes within (on the diagonal) and among (above the diagonal) black bass taxa. The abbreviation ALSPB is used for Alabama spotted bass.

	M. salmoides	*M. floridanus*	*M. dolomieu*	ALSPB	*M. punctulatus*	*M. coosae*	*M. cataractae*	*M. notius*	*M. treculi*
M. salmoides	**0.64**	3.89	10.40	8.37	10.36	8.48	8.20	7.48	6.95
M. floridanus	–	**0.59**	9.71	8.30	9.56	8.34	7.93	7.70	6.90
M. dolomieu	–	–	**0.37**	10.28	1.02	10.41	9.80	10.48	10.20
AL spotted bass	–	–	–	**0.50**	10.10	1.60[1]/2.58[2]	8.20	8.60	8.38
M. punctulatus	–	–	–	–	**0.14**	10.29	9.50	10.45	9.98
M. coosae	–	–	–	–	–	**0.60[3]/2.60[4]**	8.32	7.92	8.57
M. cataractae	–	–	–	–	–	–	**0.32**	8.57	8.32
M. notius	–	–	–	–	–	–	–	**0.46**	7.89
M. treculi	–	–	–	–	–	–	–	–	**0.14**

[1]Mean percent sequence divergence between Alabama spotted bass and *M. coosae* from Coosa River.
[2]Mean percent sequence divergence between Alabama spotted bass from Coosa River and *M. coosae* from Tallapoosa River.
[3]Mean percent sequence divergence within *M. coosae* from Coosa R and within *M. coosae* from Tallapoosa River.
[4]Mean percent sequence divergence between *M. coosae* from Coosa and *M. coosae* from Tallapoosa River.

rate variation, and unequal base composition—are accommodated.

The optimal tree for the TN+G model (as well as other models) is shown in Figure 3. All 100 random-addition-sequence replicates identified this same tree, so it seems unlikely that better trees exist. Figure 4 shows the results of a bootstrap analysis under the same model, using parameter values estimated on the optimal tree.

Searches using parsimony analysis with equal weighting found six optimal trees of length 1,492 steps. These trees were found in 99 percent of the random-addition-sequence replicates. The strict consensus of the six most parsimonious trees, along with bootstrap support values, is shown in Figure 5. These trees agree with the likelihood tree in most respects, differing only in the location at which the *Micropterus* clade is rooted and in the placement of *M. cataractae*. Apart from groups supported by low bootstrap values (=65%) using likelihood or parsimony analysis, the trees resulting from these analyses are equivalent. Other parsimony weighting schemes produced identical or similar trees, except that the *M. cataractae* is sometimes placed as the sister taxon to the clade containing *M. salmoides*, *M. floridanus*, *M. treculi*, and *M. notius* (consistent with the maximum likelihood results), albeit with low bootstrap support.

Discussion

Status of Taxa within Micropterus

The taxonomy of *Micropterus* has taken a long road of revision from its description in the early 1800s (Lacépède 1802; Henshall 1881; Jordan and Everman 1896; Hubbs and Bailey 1940; Bailey and Hubbs 1949; Ramsey 1975). All of those classifications were based on the analysis of some set of morphological characters. Using a molecular genetics approach to taxonomy, it is clear that some revision to the genus is needed.

The most substantial taxonomic finding of this study is that *M. salmoides* and *M. floridanus* are distinct and deserve recognition as separate species. The discriminant function analysis of the morphological data reliably assigned all of the *M. salmoides* to a species group and all but two of the *M. floridanus* to a different species group when all functions were analyzed. In addition, *M. floridanus* and *M. salmoides* have fixed allelic differences at several allozyme loci and have different ND-3/4 haplotypes. Haplotype differences between the taxa were also observed in an earlier study that used a different technique with different populations (Nedbal and Philipp 1994). The mtDNA sequence divergence between *M. floridanus* and *M. salmoides* was 3.89 percent, a value more than threefold greater than that observed between *M. dolomieu* and *M. punctulatus* (1.20%) and well above that observed among many other species of fish (Near et al., in press).

Another significant finding is the high level of divergence observed between the two currently recognized subspecies of *M. punctulatus*. The two forms are geographically separated and ecologically different (Vogele 1975). The range of the Alabama spotted bass is restricted to the Mobile Bay system in Alabama, Georgia, and Mississippi, whereas the range of *M. punctulatus* extends throughout much of the Ohio River drainage and into the central and lower Mississippi River drainage in Missouri, Kansas, Oklahoma, Arkansas, Louisiana, and Mississippi (Hubbs and Bailey 1940; Gilbert 1973). *Micropterus punctulatus* is widely distributed among many different stream habitats (Brown 1931; Howland 1932; Hall 1951; Lawrence 1954; Trautman 1957; Cross 1967; and Vogele 1975); the Alabama spotted bass prefers the clear lakes and rivers within the Mobile Bay drainage (Gilbert 1973). The two taxa are morphologically and genetically distinguishable as well (e.g., fixed allelic differences at multiple gene loci, fixed haplotype differences, and a sequence divergence of 10.28%). That level of divergence would indicate that the Alabama spotted bass is, in fact, not closely related to *M. punctulatus*. Because the analyses of the Alabama spotted bass were all conducted using a single collection from one source, however, the taxonomy of these two groups will remain unchanged for the time being.

Although *M. dolomieu* from several locations were analyzed, samples were collected from only one locality within the historical range of *M. d. velox*. Because preliminary analyses did not reveal differences between the two geographic sources, the status of the Neosho smallmouth bass *M. d. velox* remains to be explored. Stark and Echelle (1998), using allozymes, found evidence for three different clades of *M. dolomieu* inhabiting the interior highlands of Missouri, Arkansas, and Oklahoma in an area known as the Ozark and Ouachita uplands. Further analysis including these populations and using multiple molecular techniques needs to be completed before a revision of the taxonomy of *M. dolomieu* can be considered complete.

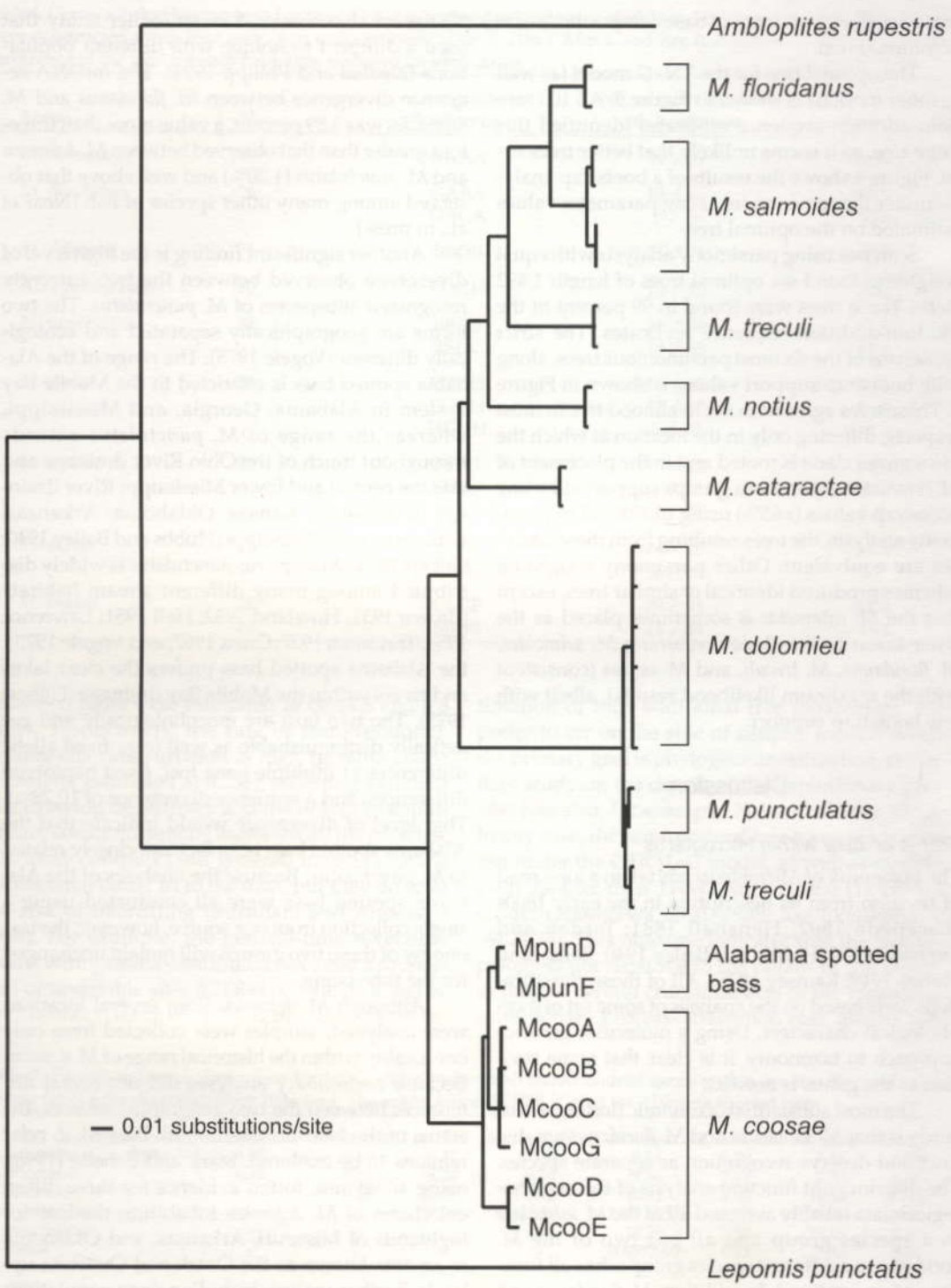

Figure 3. Maximum likelihood tree under the TN+G model for the 30 *Micropterus* cyt b and ND2 mtDNA sequences. The tree was rooted using two centrarchid outgroups. Branch lengths are proportional to the expected number of nucleotide substitutions between adjacent nodes.

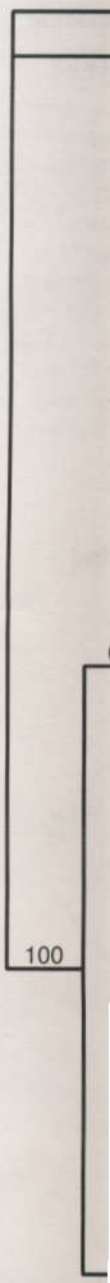

Figure 4. Bo
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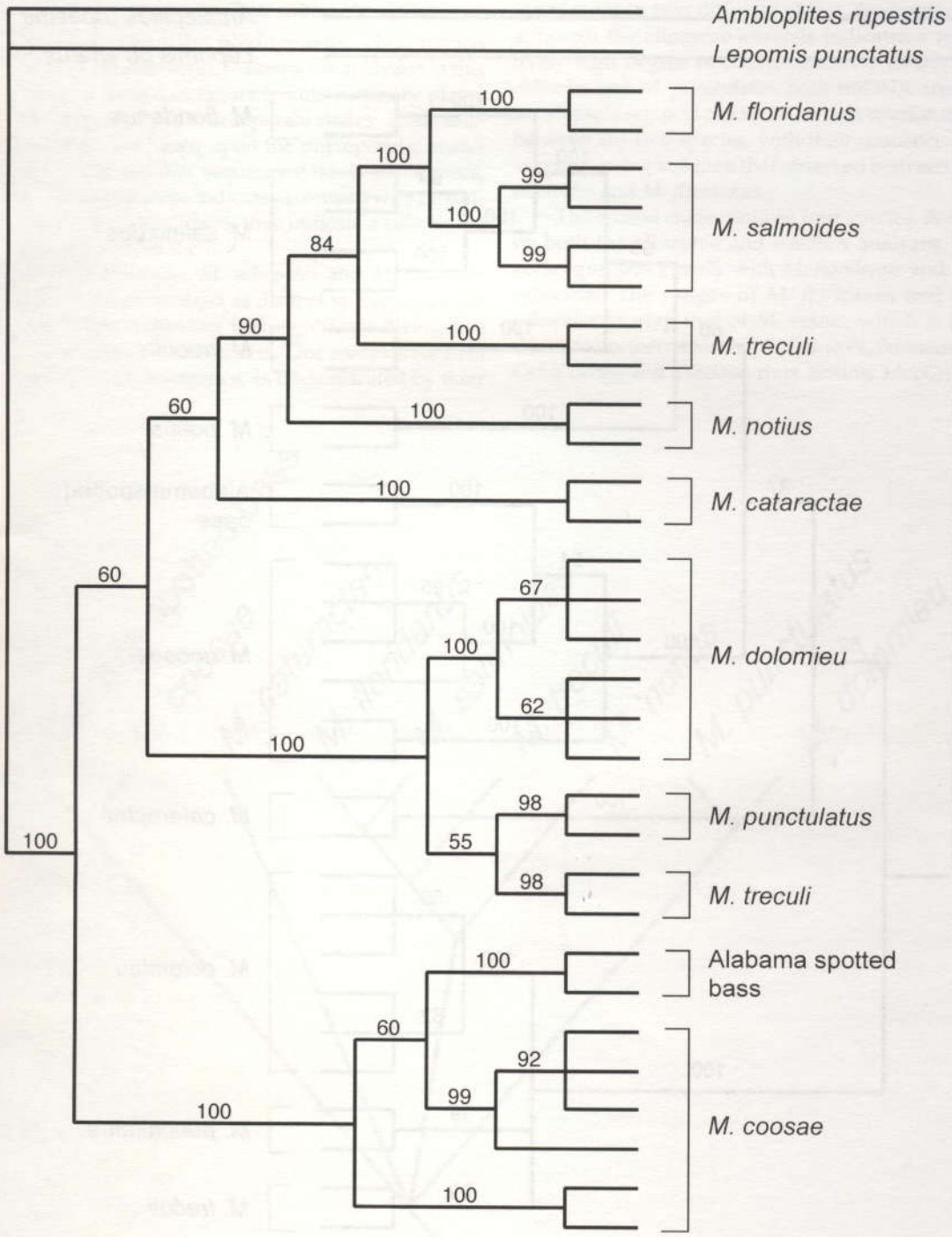

Figure 4. Bootstrap majority-rule consensus tree for maximum-likelihood analysis of mtDNA sequence data under the TN+G model. Values along branches indicate the percentage of 100 bootstrap replicates containing the corresponding clade.

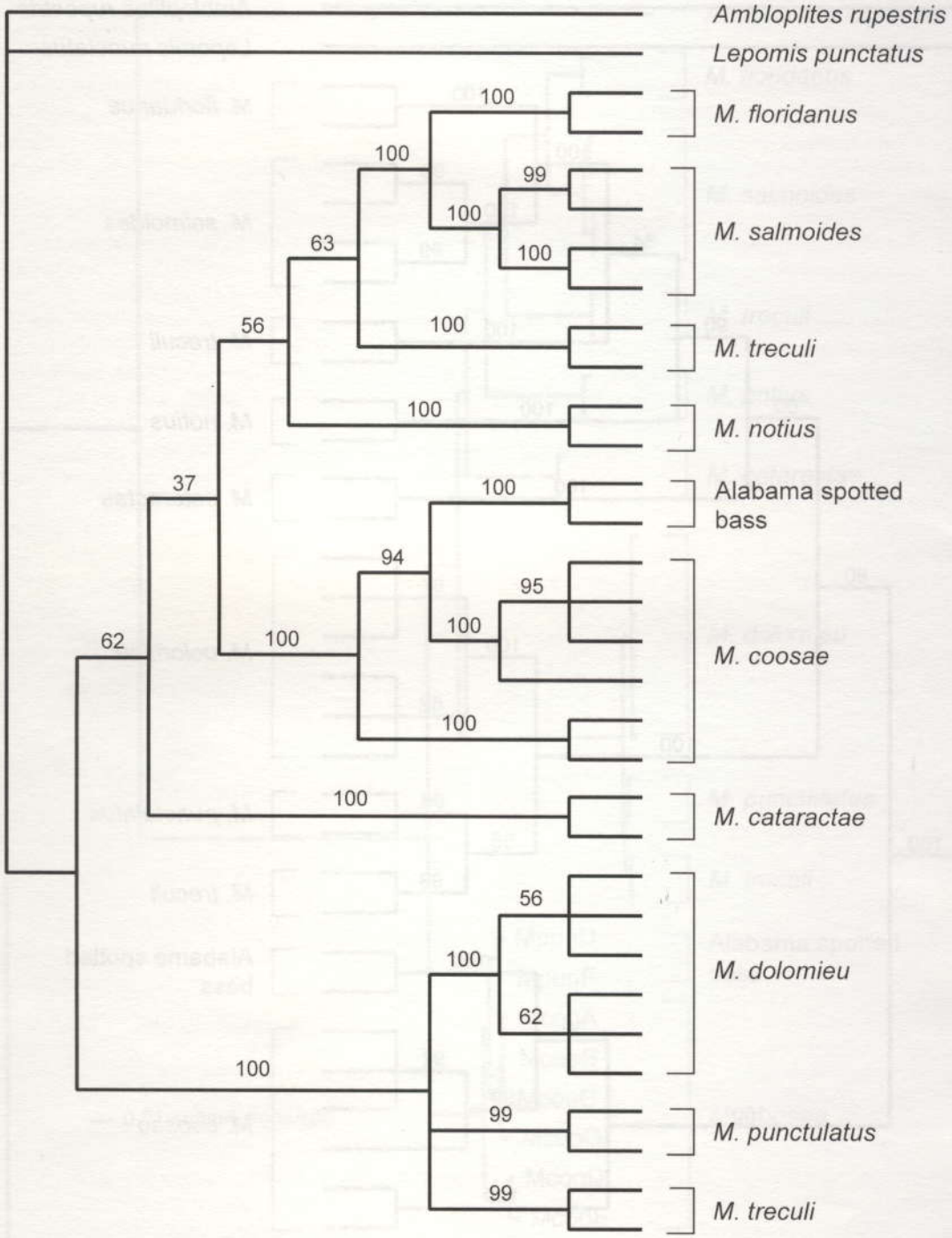

Figure 5. Strict consensus of six equally parsimonious trees under equal weighting; length = 1,492 steps. Values along branches indicate the percentage of 1,000 bootstrap replicates containing the corresponding clade.

Relationships Among Taxa within the Genus

A summary of the phylogenetic relationships among the nine taxa of *Micropterus* analyzed in this study is shown in Figure 6. This summary places the nine taxa into four separate clades. These relationships are based upon the phylogenetic analyses of the mtDNA sequence data. In some cases, these relationships indicate agreement with historical ideas, but in others, they indicate a substantial departure from them.

For example, *M. salmoides* and *M. dolomieu* have been recognized as distinct species since the late 1800s, indicating that significant divergence exists between the two taxa. Our analyses confirm that level of divergence, as demonstrated by their assignment to two different clades. Interestingly, although the allozyme analysis indicates a relatively high degree of differentiation between *M. dolomieu* and *M. punctulatus*, both mtDNA analyses strongly support a close sister taxa relationship between the two species, with their sequence divergence being less than that observed between *M. salmoides* and *M. floridanus*.

The second clade contains four species. Based on both the allozyme and mtDNA analyses, *M. notius* grouped closely with *M. floridanus* and *M. salmoides*. The ranges of *M. floridanus* and *M. salmoides* overlap that of *M. notius*, which is endemic to northern Florida (the Sante Fe, Suwannee, Ochlocknee and Wacissa river basins; MacCrim-

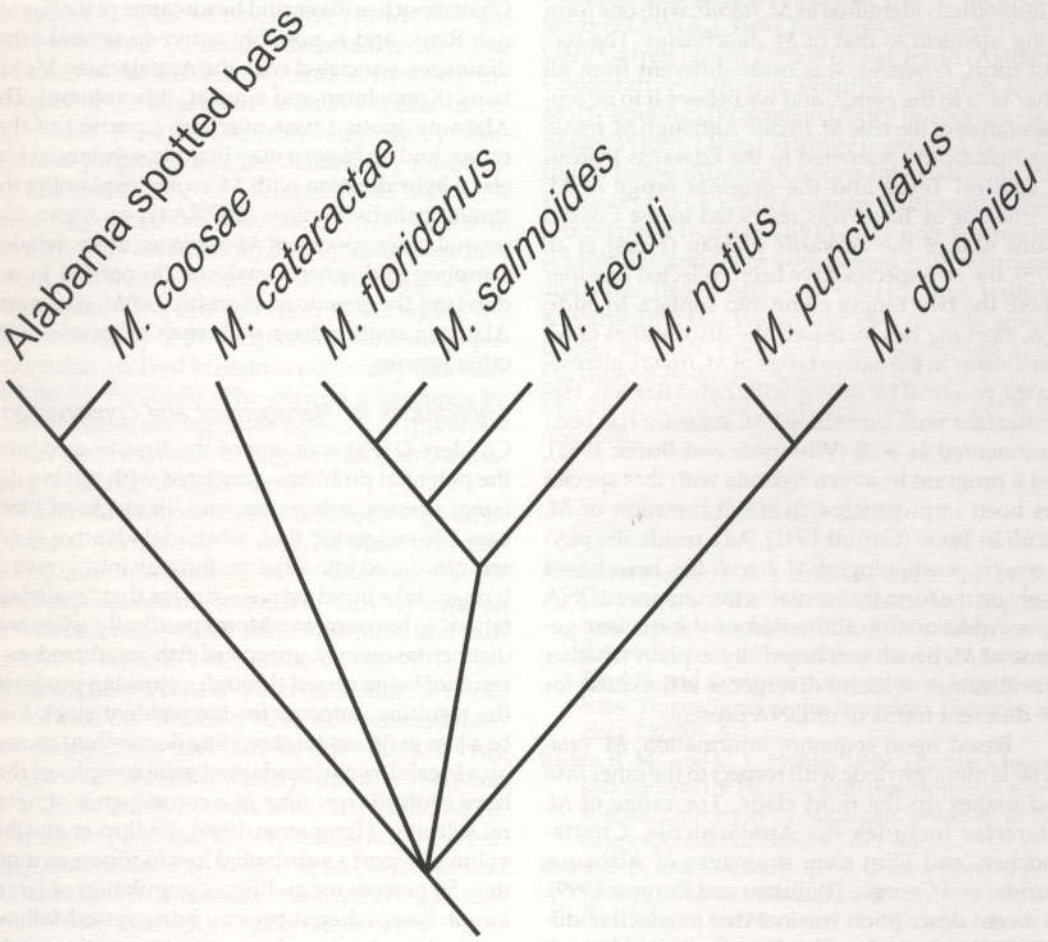

Figure 6. Summary of phylogenetic relationships containing groupings that are well supported in either the parsimony or maximum-likelihood analyses of mtDNA sequence data. The two methods of analysis agree in all aspects shown here, and any further resolution using either method is only weakly supported by bootstrapping.

mon and Robbins 1975; Koppelman and Garrett, this volume). Grouping *M. notius* in a clade containing both *M. floridanus* and *M. salmoides* is consistent with its description (Bailey and Hubbs 1949), which states that *M. notius* is structurally closest to the genus *Huro* (now recognized as *Micropterus salmoides*).

The relationship of *M. treculi* to the rest of the clade containing M. *floridanus, M. salmoides, and M. notius* is less predictable. Its overall appearance is more similar to *M. punctulatus*, and there were no fixed allelic differences between *M. treculi* and *M. punctulatus* using allozyme analysis, suggesting either that the two species have only recently diverged or that they may have hybridized. The mtDNA data support this latter conclusion; two very different mtDNA haplotypes were detected in individuals identified as *M. treculi*, with one form being identical to that of *M. punctulatus*. The second form, however, was quite different from all other taxa in the genus, and we believe it to be representative of the true *M. treculi*. Although *M. treculi* was historically restricted to the Edwards Plateau of Central Texas and the original range of *M. punctulatus* in Texas was restricted to the Coastal Plains east of the Edwards plateau (Hurst et al. 1975), the two species have been collected together where the two ranges come into contact. In addition, stocking has increased the distribution of *M. punctulatus* in the native range of *M. treculi*, increasing the potential for interspecific hybridization. Hybridization with introduced *M. dolomieu* has been documented as well (Whitmore and Butler 1982), and a program to screen hybrids with that species has been implemented in the restoration of *M. treculi* in Texas (Garrett 1991). As a result, the phylogenetic positioning of *M. treculi* has been based solely on the form that contains the unique mtDNA type. Additional examination of the nuclear genome of *M. treculi* will hopefully explain whether hybridization or recent divergence is the cause for the different forms of mtDNA present.

Based upon sequence information, *M. cataractae* is monophyletic with respect to the other taxa and makes up the third clade. The range of *M. cataractae* includes the Apalachicola, Chattahoochee, and Flint river drainages of Alabama, Florida, and Georgia, (Williams and Burgess 1999). Its recent description was not due to a lack of differentiation from the other taxa; to the contrary, it had long been considered a separate taxon and was known as the "Flint River form" of *M. coosae*, although *M. cataractae* has been the subject of controversy since its discovery in 1933 (Ramsey 1975). The meristic analysis indicated that this species is identifiable, with occasional confusion with *M. coosae* and *M. notius* possible, and it occupies a range that is adjacent to the ranges of those species.

Micropterus coosae and the Alabama spotted bass make up the fourth clade. It is interesting that the Alabama spotted bass is most closely related to *M. coosae* and only distantly related to *M. punctulatus*, with which it has been taxonomically linked for many years (Hubbs and Bailey 1940). It is also interesting that *M. coosae* was the most variable taxon according to allozyme and RFLP analysis and was polyphyletic based on mtDNA sequences. The level of differentiation observed in *M. coosae* suggests significant intraspecific genetic structuring among populations. The species has a relatively large range, which includes the Mobile Basin, the Chattahoochee River, and headwaters of the Savannah River, and is possibly native to several other drainages associated with the Appalachian Mountains (Koppelman and Garrett, this volume). The Alabama spotted bass overlaps a portion of that range, and its history may include substantial levels of hybridization with *M. coosae*, explaining the similarity between their mtDNA types. Again, like several other species of *Micropterus*, more detailed sampling and genetic analyses are needed to understand the genetic relationships of *M. coosae* and Alabama spotted bass with each other and with other species.

Implications for Management and Conservation

Childers (1975) was one of the first to recognize the potential problems associated with mixing different species, subspecies, and/or stocks of black bass. He suggested that, when maladaptive genes are introduced into a species through introgression, it might take hundreds of years for that "maladaptation" to be overcome. More specifically, when two distinct taxonomic groups of fish interbreed as a result of being mixed through a stocking program, the resulting outcome for the resident stock may be a loss in fitness (outbreeding depression) caused by a breakdown of coadapted gene complexes that have evolved over time as a consequence of natural selection (Templeton 1986). Philipp et al. (this volume) report a substantial loss in fitness of more than 50 percent for an Illinois population of largemouth bass, when it became introgressed following the introduction of two other non-native stocks of *M. salmoides* and one stock of non-native *M. floridanus*. Unfortunately, there are few fish data sets that provide insight into how distantly related two taxa (species, subspecies, stocks, or even popula-

tions) must be before a fitness loss will occur. As a result, to be able to manage bass responsibly (i.e., in a manner that protects the genetic resources of these valuable fish), we need to know the genetic structure of the operational units involved. Clearly, mixing allopatric species, and thereby enhancing interspecific hybridization, is the most potentially damaging mode of action. So, it seems logical that the first step toward responsible management would be to determine which entities are in fact distinct species. A prime example in *Micropterus* is *M. salmoides* and *M. floridanus*. Many fisheries management agencies have been treating these two species as interchangeable. Many take the approach that by introducing *M. floridanus* genes into local populations of native *M. salmoides*; there will be some "improvement" to those local populations. Philipp et al. (this volume) have shown that improvement is not the result—the actual result is loss in fitness of the resident population. In reality, the integrity of the largemouth bass as a species is being eroded at an unacceptably high rate by management programs that are deliberately introducing the non-native *M. floridanus* into native *M. salmoides* populations. Because the two species do not mate fully assortatively, F1 individuals are produced, and those individuals serve to facilitate introgression. This introgression is damaging the genetic integrity of the recipient *M. salmoides* populations that evolved in regions previously uninhabited by *M. floridanus*. The general acceptance by many fisheries management agencies of this widespread genetic erosion of largemouth bass populations is difficult to understand.

Unfortunately, similar situations exist for other species of *Micropterus*, as well. As previously discussed, the introduction of *M. dolomieu* into *M. treculi* populations has resulted in fairly widespread hybridization (Edwards 1979; Whitmore and Butler 1982; Whitmore 1983; Morizot et al. 1991). Fortunately, current conservation efforts for *M. treculi* are focused on "undoing" that management decision (Koppelman and Garrett, this volume). Why are agencies concerned with hybridization between *M. dolomieu* and *M. punctulatus*, *M. treculi* and *M. dolomieu*, or *M. coosae* and *M. cataractae*, yet seemingly unconcerned with mixing *M. floridanus* and *M. salmoides*? It is because management agencies believe that, through stocking *M. floridanus*, there will be some increase in the size of creeled fish, an endpoint that is not only extremely risky to the genetic integrity of native populations, and therefore inappropriate policy, but that is also highly unlikely (Philipp et al, this volume). Fisheries management agencies need to develop a greater concern for conserving our native resources, not only for *Micropterus*, but for other species as well. This level of management responsibility would certainly require a change in philosophy and policy among agencies, but it would also help change a public that believes only the very largest fish hold merit. If we are to stop the widespread erosion of the genetic resources of our precious populations of *Micropterus*, however, that change is not only imperative, but also long overdue.

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Appendix 1. Summary of meristics. Means ± SD (range) are given for all 14 characters for each taxon.

	Scales on lateral line	Scales above lateral line	Scales below lateral line	Scales around caudal peduncle	Scale rows on cheek	Scales before dorsal fin	Scales on opercule
M. salmoides	62.5 ± 2.8 (55–66)	7.40 ± 0.7 (7–8)	14.6 ± 1.0 (13–17)	27.9 ± 1.0 (26–30)	10.1 ± 1.0 (9–13)	26.6 ± 2.1 (22–30)	63.0 ± 10.1 (45–83)
M. floridanus	71.3 ± 3.9 (66–76)	8.3 ± 0.9 (7–10)	16.9 ± 1.1 (15–21)	30.9 ± 1.3 (28–33)	11.6 ± 1.1 (10–14)	28.6 ± 2.8 (24–35)	70.0 ± 13.6 (41–103)
M. dolomieu	72.6 ± 3.7 (64–79)	12.5 ± 1.2 (10–15)	19.8 ± 3.0 (16–32)	29.2 ± 1.7 (26–33)	16.1 ± 1.7 (13–20)	38.0 ± 3.9 (32–48)	68.4 ± 20.5 (42–110)
AL spotted bass	73.7 ± 2.1 (70–79)	9.2 ± 0.8 (8–11)	18.3 ± 1.7 (15–22)	28.8 ± 0.9 (27–31)	14.8 ± 1.4 (12–18)	34.6 ± 2.7 (31–39)	69.9 ± 6.5 (61–86)
M. punctulatus	63.6 ± 2.7 (58–71)	8.4 ± 0.8 (7–10)	14.3 ± 1.3 (11–17)	25.0 ± 1.1 (21–27)	13.3 ± 1.5 (10–17)	29.2 ± 3.3 (23–40)	53.9 ± 8.3 (29–72)
M. coosae	69.0 ± 4.0 (58–77)	9.2 ± 1.0 (7–13)	16.1 ± 1.9 (11–21)	27.7 ± 1.4 (24–31)	12.8 ± 1.5 (8–16)	34.9 ± 4.4 (10–45)	64.6 ± 10.1 (26–84)
M. cataractae	72.5 ± 3.3 (65–79)	9.6 ± 0.8 (8–12)	19.0 ± 1.5 (17–24)	31.1 ± 1.6 (27–34)	13.9 ± 1.7 (11–18)	38.4 ± 3.5 (30–44)	75.8 ± 9.3 (57–96)
M. notius	61.6 ± 2.1 (57–65)	7.7 ± 0.7 (6–9)	16.5 ± 1.1 (14–19)	28.8 ± 1.1 (27–31)	11.6 ± 1.2 (9–14)	30.2 ± 3.0 (25–37)	75.9 ± 6.9 (62–92)
M. treculi	63.4 ± 3.2 (55–68)	8.7 ± 0.8 (7–10)	16.0 ± 1.2 (14–20)	26.8 ± 1.4 (24–29)	13.2 ± 1.7 (10–18)	33.2 ± 3.0 (28–40)	61.4 ± 7.9 (45–82)

	Scales on subopercular	Scales on interopercular	# of anal rays	# of dorsal rays	Spinous/soft dorsal sep	# of pectoral rays	# of gill rakers
M. salmoides	16.5 ± 5.3 (9–28)	8.1 ± 2.0 (5–13)	11.8 ± 0.9 (10–14)	13.7 ± 0.6 (13–15)	2.2 ± 1.2 (1–5)	14.3 ± 0.6 (13–15)	7.9 ± 0.5 (7–9)
M. floridanus	20.8 ± 5.1 (10–29)	9.4 ± 3.4 (2–19)	11.7 ± 0.5 (3–12)	13.4 ± 0.9 (10–15)	2.1 ± 0.9 (1–4)	14.5 ± 1.2 (9–16)	7.8 ± 0.7 (6–9)
M. dolomieu	14.2 ± 3.4 (6–21)	10.3 ± 3.8 (5–19)	11.1 ± 0.6 (10–12)	13.3 ± 1.0 (10–15)	4.6 ± 1.6 (1–10)	15.7 ± 1.0 (13–17)	7.3 ± 0.6 (6–9)
AL spotted bass	16.4 ± 3.1 (12–25)	7.7 ± 1.3 (5–10)	11.0 ± 0.2 (11–12)	13.0 ± 0.2 (12–13)	3.8 ± 1.3 (2–6)	15.3 ± 0.8 (14–16)	7.4 ± 0.7 (6–8)
M. punctulatus	11.2 ± 2.5 (6–17)	6.7 ± 1.3 (3–11)	10.2 ± 0.6 (9–12)	12.1 ± 0.6 (11–14)	3.4 ± 1.2 (1–6)	15.4 ± 0.8 (13–17)	6.3 ± 0.8 (3–7)
M. coosae	13.7 ± 3.1 (9–25)	7.9 ± 1.5 (4–12)	10.1 ± 0.3 (9–11)	12.1 ± 0.5 (11–14)	3.9 ± 1.3 (1–11)	15.1 ± 0.7 (13–17)	6.6 ± 0.9 (4–8)
M. cataractae	19.0 ± 3.6 (11–26)	9.8 ± 2.2 (7–16)	10.1 ± 0.3 (10–11)	12.1 ± 0.3 (12–13)	5.4 ± 3.2 (2–12)	16.2 ± 0.5 (15–17)	6.6 ± 0.8 (5–8)
M. notius	19.6 ± 4.1 (12–28)	9.6 ± 2.5 (4–18)	10.9 ± 1.0 (10–14)	12.4 ± 1.0 (9–14)	7.4 ± 2.6 (2–12)	15.9 ± 0.4 (15–17)	5.8 ± 0.8 (5–7)
M. treculi	12.2 ± 3.1 (8 –23)	6.9 ± 1.6 (4–10)	10.0 ± 0.4 (9–11)	12.1 ± 0.4 (11–13)	4.2 ± 1.4 (2–8)	14.5 ± 1.0 (12–16)	7.2 ± 0.7 (5–8)

Appendix 2. Allele frequencies for the different taxa (36 populations) of black bass analyzed for 19 polymorphic loci. Sample sizes for each population are listed in Table 2. The

Appendix 2. Allele frequencies for the different taxa (36 populations) of black bass analyzed for 19 polymorphic loci. Sample sizes for each population are listed in Table 2. The allelic designations are based on relative mobility, with 100 representing the slowest allele detected among all taxa.

	Taxa/population number														
	M. salmoides					*M. floridanus*					*M. dolomieu*				
Locus	1	2	3	4	5	8	9	10	11	12	13	14	15	16	17
*sAAT-A**															
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
122	1.000	1.000	1.000	1.000	1.000	0.950	0.900	0.900	0.975	0.825	1.000	1.000	1.000	1.000	1.000
130	0.000	0.000	0.000	0.000	0.000	0.050	0.100	0.100	0.025	0.175	0.000	0.000	0.000	0.000	0.000
sAAT-B*															
100	1.000	0.933	1.000	0.525	0.450	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
110	0.000	0.067	0.000	0.475	0.550	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
126	0.000	0.000	0.000	0.000	0.000	0.750	0.450	0.550	0.675	0.800	1.000	1.000	1.000	1.000	1.000
139	0.000	0.000	0.000	0.000	0.000	0.250	0.550	0.450	0.325	0.200	0.000	0.000	0.000	0.000	0.000
ADH*															
–100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
–160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CBP*															
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000
103	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
105	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
108	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CK-B*															
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.975	1.000	1.000	1.000	1.000	1.000	1.000
105	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000
CK-C*															
100	0.050	0.000	0.025	0.025	0.100	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.075	0.000
135	0.950	1.000	0.975	0.975	0.900	0.983	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.925	1.000
194	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*EST**															
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
102	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
106	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Appendix 2. Continued.

	Taxa/population number															
	M. salmoides					*M. floridanus*					*M. dolomieu*					
Locus	1	2	3	4	5	8	9	10	11	12	13	14	15	16	17	
*G3PDH**																
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
154	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
*GPI-A**																
100	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
115	1.000	1.000	1.000	1.000	1.000	1.000	0.975	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
GPI-B*																
100	0.925	1.000	1.000	1.000	1.000	0.967	1.000	1.000	1.000	1.000	1.000	0.967	1.000	1.000	1.000	
144	0.075	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000	
*sIDHP-B**																
100	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
120	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
140	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
LDH-A*																
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
159	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.975	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
LDH-B*																
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
122	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
LDH-C*																
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.517	0.233	0.750	0.625	0.600	
102	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
105	1.000	1.000	1.000	1.000	1.000	1.000	0.975	1.000	1.000	1.000	0.483	0.767	0.250	0.375	0.400	
sMDH-A*																
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	

Appendix 2. Continued.

Appendix 2. Continued.

	Taxa/population number															
	M. salmoides					*M. floridanus*					*M. dolomieu*					
Locus	1	2	3	4	5	8	9	10	11	12	13	14	15	16	17	
146	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.975	1.000	1.000	1.000	1.000	1.000	1.000	
182	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
sMDH-B*																
100	0.925	0.933	1.000	0.575	0.925	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
114	0.075	0.067	0.000	0.425	0.075	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	
128	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	
*PGDH**																
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
108	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
PGM*																
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
137	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	
153	1.000	1.000	1.000	0.925	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	
175	0.000	0.000	0.000	0.075	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
*SOD**																
100	0.000	0.000	0.000	0.000	0.000	0.333	0.300	0.475	0.400	0.675	0.000	0.000	0.000	0.000	0.000	
147	1.000	1.000	1.000	1.000	1.000	0.667	0.700	0.525	0.600	0.325	0.483	0.967	0.625	0.725	0.967	
212	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.517	0.033	0.375	0.275	0.033	

	Taxa / Population Number														
	M. dolomieu ALSPB		*M. punctulatus*					*M. coosae*					*M. cataractae*		
Locus	19	20	21	22	23	24	25	26	27	28	29	30	32	33	34
*sAAT-A**															
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
122	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sAAT-B*															
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
126	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.972	0.925	1.000	1.000	1.000	1.000	1.000
139	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.075	0.000	0.000	0.000	0.000	0.000

Appendix 2. Continued.

	Taxa/population number														
	M. dolomieu ALSPB		*M. punctulatus*					*M. coosae*					*M. cataractae*		
Locus	19	20	21	22	23	24	25	26	27	28	29	30	32	33	34
ADH*															
–100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.972	0.850	0.900	1.000	1.000	1.000	1.000
–160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.150	0.100	0.000	0.000	0.000	0.000
CBP*															
100	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
103	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000
105	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
108	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000
CK-B*															
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
105	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CK-C*															
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000	0.000
135	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.889	1.000	1.000	1.000	1.000	1.000	1.000
194	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000
*EST**															
100	0.000	0.925	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
102	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000
106	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000
110	0.000	0.075	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*G3PDH**															
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.050	0.000	0.000	0.000	0.000	0.000
154	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.950	1.000	0.950	1.000	1.000	1.000	1.000	1.000
*GPI-A**															
100	0.000	0.075	0.000	0.000	0.125	0.000	0.158	0.275	0.083	0.300	0.000	0.025	0.000	0.000	0.000
115	1.000	0.925	1.000	1.000	0.875	1.000	0.842	0.725	0.917	0.700	0.950	0.975	1.000	1.000	1.000
130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000
*GPI-B**															
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
144	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Appendix 2. Continued.

	Taxa/population number														
	M. dolomieu ALSPB		*M. punctulatus*					*M. coosae*					*M. cataractae*		
Locus	19	20	21	22	23	24	25	26	27	28	29	30	32	33	34
*sIDHP-B**															
100	0.000	0.175	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
110	0.000	0.000	0.825	0.983	0.988	0.588	0.974	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
120	1.000	0.825	0.000	0.000	0.000	0.000	0.000	0.775	1.000	0.725	0.650	0.525	1.000	0.958	0.800
130	0.000	0.000	0.175	0.017	0.012	0.412	0.026	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
140	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.225	0.000	0.275	0.350	0.475	0.000	0.042	0.200
LDH-A*															
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
159	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LDH-B*															
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000	0.000
122	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.944	1.000	1.000	1.000	1.000	1.000	1.000
LDH-C*															
100	0.775	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.192	0.042	0.000
102	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
105	0.225	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.808	0.958	1.000
sMDH-A*															
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
146	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.889	1.000	1.000	0.975	1.000	1.000	1.000
182	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111	0.000	0.000	0.025	0.000	0.000	0.000
sMDH-B*															
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
114	0.000	1.000	1.000	1.000	1.000	1.000	1.000	0.600	0.917	0.650	0.925	0.750	1.000	1.000	1.000
128	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.400	0.083	0.350	0.075	0.250	0.000	0.000	0.000
*PGDH**															
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.975	1.000	1.000	1.000
108	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000
PGM*															
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.975	0.883	1.000	1.000	0.750	0.000	0.000	0.000
137	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.167	0.000	0.000	0.250	0.000	0.000	0.000

Appendix 2. Continued.

	Taxa/population number														
	M. dolomieu ALSPB		*M. punctulatus*					*M. coosae*					*M. cataractae*		
Locus	19	20	21	22	23	24	25	26	27	28	29	30	32	33	34
153	0.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000
175	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*SOD**															
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000
147	0.900	1.000	1.000	1.000	1.000	1.000	1.000	0.950	1.000	1.000	1.000	1.000	1.000	1.000	1.000
212	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

	Taxa/population number					
	M. notius			*M. treculi*		
Locus	35	36	37	38	39	40
*sAAT-A**						
100	0.875	0.750	0.976	0.000	0.000	0.000
122	0.125	0.250	0.024	1.000	1.000	1.000
130	0.000	0.000	0.000	0.000	0.000	0.000
sAAT-B*						
100	0.000	0.000	0.000	0.000	0.000	0.000
110	0.000	0.000	0.000	0.000	0.000	0.000
126	1.000	1.000	1.000	1.000	1.000	1.000
139	0.000	0.000	0.000	0.000	0.000	0.000
ADH*						
-100	1.000	1.000	1.000	1.000	1.000	1.000
-160	0.000	0.000	0.000	0.000	0.000	0.000
CBP*						
100	0.000	0.000	0.000	0.000	0.000	0.000
103	1.000	1.000	1.000	1.000	1.000	1.000
105	0.000	0.000	0.000	0.000	0.000	0.000
108	0.000	0.000	0.000	0.000	0.000	0.000
CK-B*						
100	1.000	1.000	1.000	1.000	1.000	1.000
105	0.000	0.000	0.000	0.000	0.000	0.000

Appendix 2. Continued.

	Taxa/population number					
	M. notius			*M. treculi*		
Locus	35	36	37	38	39	40
CK-C*						
100	0.050	0.000	0.000	0.000	0.000	0.000
135	0.950	1.000	1.000	1.000	0.875	1.000
194	0.000	0.000	0.000	0.000	0.125	0.000
*EST**						
100	0.000	0.000	0.000	0.000	0.000	0.000
102	0.000	0.000	0.000	0.000	0.000	0.000
106	1.000	1.000	1.000	0.000	0.000	0.000
110	0.000	0.000	0.000	1.000	1.000	1.000
*G3PDH**						
100	0.000	0.000	0.000	0.000	0.000	0.000
154	1.000	1.000	1.000	1.000	1.000	1.000
*GPI-A**						
100	0.000	0.000	0.000	0.071	0.150	0.000
115	1.000	1.000	1.000	0.929	0.850	1.000
130	0.000	0.000	0.000	0.000	0.000	0.000
*GPI-B**						
100	1.000	1.000	1.000	1.000	1.000	1.000
144	0.000	0.000	0.000	0.000	0.000	0.000
*sIDHP-B**						
100	0.000	0.000	0.000	0.000	0.000	0.000
110	0.000	0.000	0.000	1.000	1.000	1.000
120	1.000	1.000	1.000	0.000	0.000	0.000
130	0.000	0.000	0.000	0.000	0.000	0.000
140	0.000	0.000	0.000	0.000	0.000	0.000
LDH-A*						
100	0.000	0.000	0.000	0.000	0.000	0.000
159	1.000	1.000	1.000	1.000	1.000	1.000
LDH-B*						
100	0.000	0.000	0.000	0.000	0.000	0.000

Appendix 2. Continued.

	Taxa/population number					
	M. notius			*M. treculi*		
Locus	35	36	37	38	39	40
122	1.000	1.000	1.000	1.000	1.000	1.000
LDH-C*						
100	0.000	0.000	0.000	0.000	0.000	0.000
102	0.000	0.000	0.000	0.000	0.000	0.000
105	1.000	1.000	1.000	1.000	1.000	1.000
sMDH-A*						
100	0.000	0.000	0.000	0.000	0.000	0.000
146	1.000	1.000	1.000	1.000	1.000	1.000
182	0.000	0.000	0.000	0.000	0.000	0.000
sMDH-B*						
100	0.000	0.000	0.000	0.000	0.000	0.000
114	1.000	1.000	1.000	1.000	1.000	1.000
128	0.000	0.000	0.000	0.000	0.000	0.000
*PGDH**						
100	1.000	1.000	1.000	1.000	1.000	1.000
108	0.000	0.000	0.000	0.000	0.000	0.000
PGM*						
100	0.000	0.000	0.000	0.000	0.000	0.000
137	0.000	0.000	0.000	0.000	0.000	0.000
153	1.000	1.000	1.000	1.000	1.000	1.000
175	0.000	0.000	0.000	0.000	0.000	0.000
*SOD**						
100	0.000	0.000	0.000	0.000	0.000	0.000
147	1.000	1.000	1.000	1.000	1.000	1.000
212	0.000	0.000	0.000	0.000	0.000	0.000