



# Congeneric phylogeographical sampling reveals polyphyly and novel biodiversity within black basses (Centrarchidae: *Micropterus*)

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For groups of animals with limited or unstable taxonomic resolution, congeneric phylogeographical sampling represents a way to potentially increase resolution of species limits and internal branches during phylogenetic inference. We investigated species limits and whether there was better support for hypothesized relationships among *Micropterus* (black basses) using wide geographical sampling. Bayesian and maximum likelihood estimates of *Micropterus* phylogeny including 205 mitochondrial DNA cytochrome *b* (1140 bp) sequences (150 haplotypes) representing eight extant species and one subspecies recovered eight well-supported clades. Haplotypes from fish identified as *Micropterus coosae*, *Micropterus henshalli*, *Micropterus punctulatus*, *Micropterus salmoides*, and *Micropterus treculii* created eight forms of broad-sense polyphyly in the tree, which we hypothesized were the result of incomplete lineage sorting and hybridization-mediated introgression (natural and anthropogenic, i.e. associated with stocking). Our findings mostly agree with hypothesized relationships. However, they provide a more complex view of *Micropterus* biodiversity, highlighting population-level processes. Our data also provide a useful guide for expanding character sampling (nuclear loci and morphology) to evaluate the history, distinctiveness, and geographical distributions of genetic lineages within and among black basses. © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, 104, 346–363.

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## INTRODUCTION

Traditional phylogenetic sampling strategies often emphasize interspecific genetic variation at broad taxonomic scales, and use only one or a few individuals per species. By contrast, population biologists stress intraspecific genetic diversity at broader spatial scales via multiple samples of a taxon, although frequently

for only a portion of the species range. The classic problem with this dichotomy is that the assumptions adopted by systematists weaken phylogenetic inferences, whereas the limited comparative scale used by geneticists weakens their inferences of historical events. Systematists frequently make the assumption that nominal taxa are surrogates for evolutionary species (Barracough & Nee, 2001). This leads both systematists and population geneticists to treating current species limits and taxonomy as accurate representations of evolutionary reality, rather than hypotheses to be tested. A wealth of literature in systematics and phylogeography questions this practice (references in Avise, 2000; Hey, 2001; Funk &

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Omland, 2003; Avila, Morando & Sites, 2006). For example, low-dispersal species are often characterized by high genealogical structuring in the form of geographically concordant genetic breaks across taxa from the same area, and they sometimes contain cryptic species (Avise, 2000; Bickford *et al.*, 2007). Also, phylogenetic studies that subsample species assume nominal taxa are monophyletic for the characters sampled. In their widely cited review, Funk & Omland (2003) showed that this was often a false assumption: polyphyly is a general feature present in 23% of mitochondrial DNA (mtDNA) gene trees taken from animal literature. The same study also showed that limited spatial (within-taxon) sampling in systematics not only reduces the probability of recovering polyphyly *s.l.* (including paraphyly; see discussion in Funk & Omland, 2003), but may also compromise the accuracy of phylogenetic and demographic inferences. For example, unrecognized polyphyly can lead to systematic error, or erroneous evolutionary rates, divergence patterns, and trait distributions.

In response to these issues with phylogenetic sampling, Funk & Omland (2003) called for 'congeneric phylogeography', a merger between phylogenetic and population genetic sampling methods to improve inferences in both fields. This concept encouraged systematists to sample comprehensively at the phylogeographical scale (spatially) within each taxon when studying relationships among congeners. It spurred multi-species taxon delimitation with expanded sampling (e.g., Avila *et al.*, 2006) and bolstered opposition to DNA barcoding (Brower, 2006). However, largely because the relative value of taxon versus character sampling has dominated recent discussions (see reviews in Zwickl & Hillis, 2002; Rokas & Carroll, 2005; Heath, Hedtke & Hillis, 2008), phylogeographical sampling above the intraspecific level has received scant attention in systematics. This is strange because theory predicts that, despite increasing homoplasy at some points along a topology (e.g. species lineages), congeneric phylogeography should increase the solution space and systematic accuracy (Funk & Omland, 2003; Heath *et al.*, 2008). Also, phylogeography has repeatedly shown that mtDNA gene trees based on wide sampling can determine clades for subsampling in subsequent nuclear gene analyses (Morando *et al.*, 2004; Schönuth *et al.*, 2008; Schönuth & Mayden, 2010). Thus, congeneric phylogeographical sampling can uncover the appropriate path for project design. Finally, estimates of times to the most recent common ancestors (TMRCA) of intra- and interspecific lineages have become increasingly popular in molecular systematics and evolution. Increased numerical and spatial within-taxon sampling via congeneric phylogeography allows additional estimates of within-taxon coales-

cences, and may produce better TMRCA and phylogeny estimates from coalescent-based methods (Drummond *et al.*, 2006). In practice, we expect better coalescent time estimates primarily within clades; however, whether and to what degree changes in interclade (specific) divergence times result will depend on whether a given topology is altered by the discovery of new clades. At least for these reasons, systematists should take greater interest in congeneric phylogeographical sampling.

There are several fruitful ways forward for applying congeneric phylogeographical sampling in systematics. For example, ideal candidate taxa would be groups of animals with limited or unstable taxonomic resolution, particularly those in which the taxonomy has been dominated by morphological descriptions. Other ideal groups would be those for which molecular systematic analyses show limited gains in resolving internal branches by increasing character sampling.

We investigated the impact of congeneric phylogeographical sampling on *Micropterus* ('black bass,' Teleostei: Centrarchidae) systematics. *Micropterus* has eight recognized species and one subspecies (Table 1; Lee *et al.*, 1980; Eschmeyer, 2003). They are endemic North American freshwater fishes, the native range of which spanned east of the Continental Divide, south to Coahuila, Mexico, and north into Lake Huron drainages in southern Canada (MacCrimmon & Robbins, 1975; Lee *et al.*, 1980). *Micropterus* diversity is highest in the unglaciated south-eastern USA (seven species, one subspecies). Molecular phylogenetic analyses of *Micropterus* have evaluated relationships using limited spatial/taxon sampling and increasing numbers of characters. Johnson, Magee & Hodge (2001; sampling one individual per species) hypothesized that *Micropterus salmoides* is basal using mtDNA restriction endonuclease data. This agreed with morphology-based hypotheses (Fig. 1; Branson & Moore, 1962; Ramsey, 1975). Kassler *et al.* (2002; sampling between one and six individuals per species) broadened character sampling to include meristics, allozymes, and mtDNA cytochrome *b* (*cytb*) and NADH subunit 2 (*ND2*) sequence data, and recovered four *Micropterus* clades with unresolved internal branches (Fig. 1). Their hypotheses showed *Micropterus treculii* to be polyphyletic, differed from Ramsey's (1975) morphological hypothesis considering *Micropterus coosae* closely related to *Micropterus dolomieu*, and conflicted with Johnson *et al.*'s (2001) basal placement of *M. salmoides* (Fig. 1). Near *et al.* (2003; 50 sites, sampling between two and ten individuals per species) studied *Micropterus* relationships, Near, Bolnick & Wainwright (2004; sampling one or two individuals per species) conducted the first multilocus analyses of

**Table 1.** Taxonomy of *Micropterus*

Taxon name	Common name	Status
<i>Micropterus cataractae</i> Williams & Burgess, 1999	Shoal Bass	Valid
<i>Micropterus coosae</i> Hubbs & Bailey, 1940	Redeye Bass	Valid
<i>Micropterus dolomieu</i> Lacepède, 1802	Smallmouth Bass	Valid
<i>Micropterus henshalli</i> Hubbs & Bailey, 1940	Alabama Bass	Valid; originally described as a subspecies of <i>M. punctulatus</i> ; elevated to species status (Baker <i>et al.</i> , 2008)
<i>Micropterus notius</i> Bailey & Hubbs, 1949	Suwannee Bass	Valid
<i>Micropterus punctulatus</i> (Rafinesque, 1819)	Spotted Bass	Valid
<i>Micropterus salmoides</i> (Lacepède, 1802)	Largemouth Bass	Valid
<i>Micropterus salmoides floridanus</i> (Lesueur, 1822)	Florida Largemouth Bass	Valid as subspecies described by Bailey & Hubbs (1949), for example see Nelson <i>et al.</i> (2004); however, others have recommended species rank (Kassler <i>et al.</i> , 2002; Near <i>et al.</i> , 2003)
<i>Micropterus treculii</i> (Vaillant & Bocourt, 1874)	Guadalupe Bass	Valid
' <i>Micropterus punctulatus wichitae</i> ' Hubbs & Bailey, 1940	'Wichitae Spotted Bass'	Synonym of <i>Micropterus</i> ; discredited as <i>M. dolomieu</i> × <i>M. punctulatus</i> hybrid (Cofer, 1995)
' <i>Micropterus dolomieu velox</i> ' Hubbs & Bailey, 1940	'Neosho Smallmouth Bass'	Synonymized with <i>M. dolomieu</i> ; thought to represent clinal <i>M. dolomieu</i> morphological variation (Gilbert, 1998)

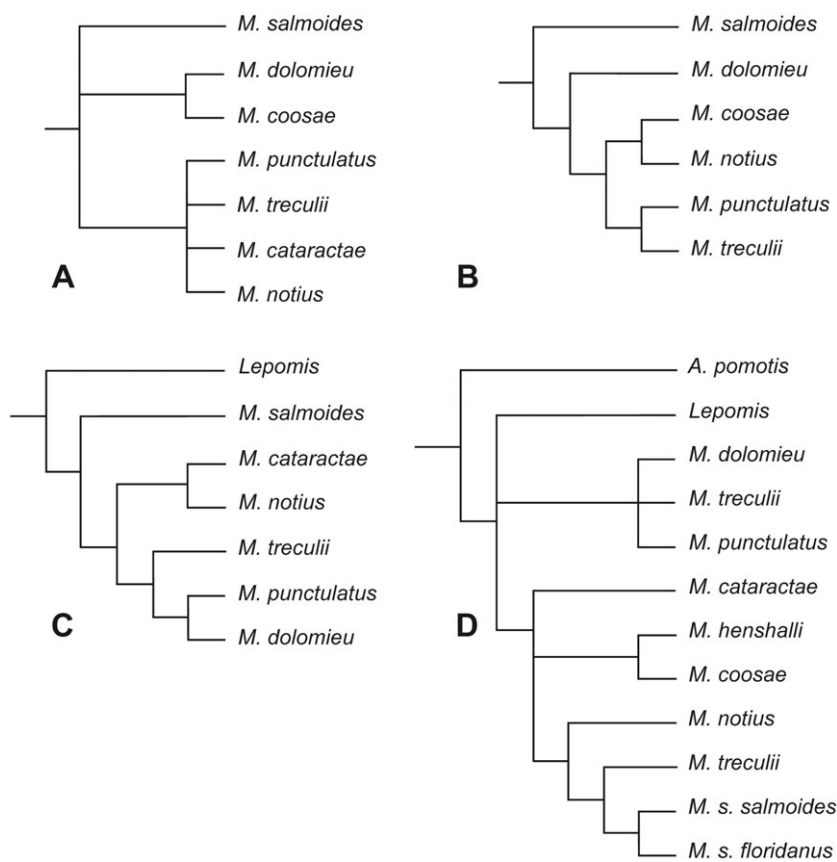
*Micropterus*, and a subsequent study (Near, Bolnick & Wainwright, 2005; sampling one individual per species) further augmented character sampling. Topologies recovered by these recent studies were essentially identical, despite Near *et al.*'s (2005) two-fold increase in characters up to 5553 bp (nucleotide base pairs) relative to Kassler *et al.*'s (2002) 2187-bp alignment (Fig. 1).

Increases in character sampling have been ineffective at resolving internal branching of the *Micropterus* phylogeny. This suggests more informative DNA characters are needed to resolve interior nodes. However, this also shows *Micropterus* spp. as being ideal for applying congeneric phylogeographical sampling to enlighten our view not only of interrelationships, but also of species limits and phylogeography. In light of the discussion above, our study may benefit *Micropterus* systematics in several ways. We predicted expanded sampling would allow us to uncover more topological polyphyly *s.l.*, potentially suggesting population-level processes relevant to macroevolution. Beyond population structure data relevant for stock management (genetic diversity and hybridization data, e.g., Barwick *et al.*, 2006), a phylogeographical perspective is broadly lacking for *Micropterus* (but see Kassler *et al.*, 2002). Thus, by expanding sampling we shed new light on geographical distributions of *Micropterus* genetic lineages, including novel biodiversity patterns.

## MATERIAL AND METHODS

### SAMPLING AND DNA SEQUENCE DATA

We collected fishes using seine or electrofishing techniques, and specimens (whole or tissue samples) were stored in 95% EtOH at between  $-80^{\circ}\text{C}$  and  $-23^{\circ}\text{C}$ . Our sampling strategy targeted one or more individuals per population for multiple populations dispersed throughout as much of each species distribution as possible. We extracted DNA from tissues using cetyltrimethylammonium bromide (CTAB)-chloroform extractions or commercial DNA extraction kits (QIAGEN DNeasy Blood & Tissue Kit). We amplified *cytb* for 152 *Micropterus* specimens using forward and reverse primers from Song, Near & Page (1998), and the forward primer GLU31 5'-TGRCTTGAAAAA CCACCGTTGT-3' (Unmack *et al.*, 2009) and reverse primer HD/INH 5'-GGGTTGTTTGATCCTGTTTCGT-3' (Schmidt, Bielawski & Gold, 1998). PCR conditions and sequencing followed Roe, Harris & Mayden (2002), except we purified PCR products using Sephadex<sup>TM</sup> beads (GE Healthcare). We obtained sequences of additional *Micropterus* (53 sequences and 44 haplotypes) and related centrarchid outgroup taxa (32 sequences) from GenBank (Kassler *et al.*, 2002; Roe *et al.*, 2002; Near *et al.*, 2004; Harris, Roe & Mayden, 2005). This resulted in a *cytb* sequence database with 237 sequences, including 205 *Micropterus*, and a ~3.5- to 20-fold increase in the number of samples



**Figure 1.** Main phylogenetic hypotheses of relationships among *Micropterus*. The first two trees are morphological hypotheses: A, Branson & Moore (1962); B, Ramsey (1975). The second two hypotheses are from molecular studies with limited within-taxon sampling and different levels of character sampling. C, Dollo parsimony consensus from Johnson *et al.* (2001). D, consensus of *Micropterus* relationships recovered by Kassler *et al.* (2002) and Near *et al.* (2003, 2004, 2005).

relative to some molecular studies (e.g. Near *et al.*, 2003). Cytochrome *b* alignment was unambiguous, so we aligned sequences manually and checked for errors, e.g. stop codons, by translation into amino acid sequences in BIOEDIT 7.0.9 (Hall, 1999). We used TCS 1.21 (Clement, Posada & Crandall, 2000) to collapse this alignment into a final matrix with 150 *Micropterus* haplotypes (doubly counting haplotypes in two taxa/morphotypes) plus outgroups used in the following analyses (Appendix).

#### PHYLOGENETIC ANALYSES

We used JMODELTEST 1.0 (Posada, 2008) to identify the best-fit model of DNA sequence evolution with a maximum likelihood (ML)-optimized Akaike information criterion (AIC) procedure. We used MEGA 3.1 (Kumar, Tamura & Nei, 2004) to enumerate variable, parsimony informative, and conserved sites. We computed mean among-clade sequence divergences using a matrix of distances corrected with the JMODELTEST-selected substitution model, gener-

ated in PAUP\* 4.0b10 (Swofford, 2002). We performed Bayesian phylogenetic inference analyses (BIs; Larget & Simon, 1999) using MRBAYES 3.12 (Ronquist & Huelsenbeck, 2003). Each MRBAYES analysis ran for  $5 \times 10^6$  generations (four chains and five separate runs), sampling one from 100 trees and fixing appropriate model priors at values estimated by JMODELTEST. After discarding 12 500 trees as burn in, we constructed a 50% majority rule consensus tree. Maximum likelihood (ML) analyses in GARLI 0.94 (Zwickl, 2006, specifying model parameters from JMODELTEST) recovered topologies (not shown) that were similar to those from Bayesian runs. However, we compared ML bootstrap proportions (BPs) estimated using GARLI (from one run starting with four individuals,  $5 \times 10^6$  generations, 400 pseudoreplicates, and 50% majority rule consensus) with Bayesian posterior probability (PP) estimates of nodal support. We considered nodes with ML BPs > 70 and PP  $\geq$  95 to be strongly supported, and placed values  $\geq$  50% along nodes (Hillis & Bull, 1993; Wilcox *et al.*, 2002). We rooted results using *Acantharchus pomotis*



(mud sunfish) as the outgroup taxon, following Near *et al.* (2005).

# RESULTS

## DNA SEQUENCE VARIATION AND EVOLUTION

Our haplotype alignment contained 770 conserved sites (total alignment: 67.5%) and 370 variable sites (32.5%), 260 of which were parsimony informative (22.8%). JMODELTEST selected the TIM3 + I model of DNA nucleotide substitution ( $I = 0.4020$ ), with a continuous  $\Gamma$  distribution adjusted by shape parameter  $\alpha$  ( $= 0.8590$ ); this model is a special case of GTR + I +  $\Gamma$ . Pairwise sequence divergence (Table 2) from a TIM3 + I +  $\Gamma$  corrected distance matrix ranged from 1.2% (clade VIIa versus clade VIIb) to 14.4% (clade V versus clade VIIa). The average model-corrected pairwise divergence among clades/subclades was 9.8%.

## PHYLOGENETIC ANALYSES

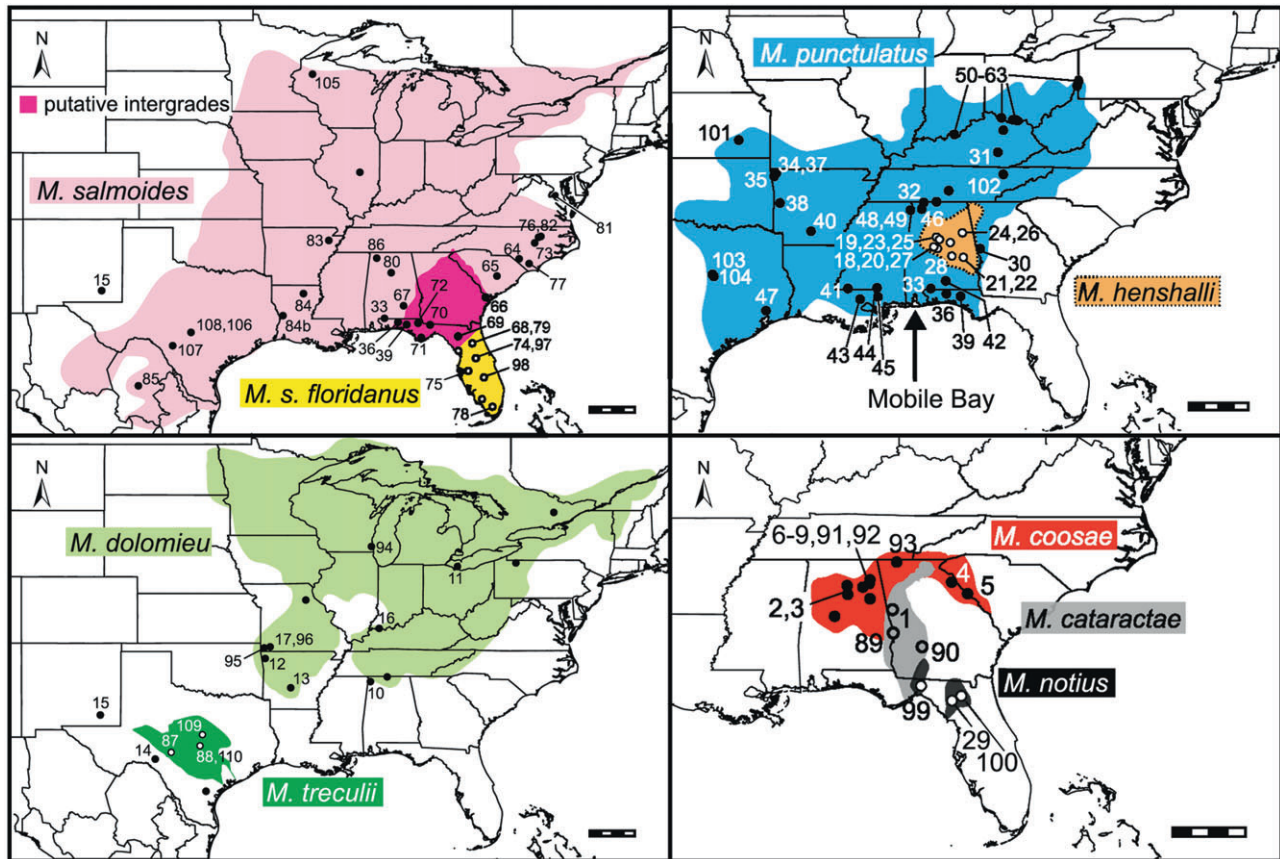
Our Bayesian phylogenetic estimate (Fig. 3) recovered *Micropterus* as monophyletic (PP/BP = 1/100), with eight moderately to strongly supported major clades (PP range = 0.99–1; BP range = 90–100). Clade I included nine *Micropterus salmoides floridanus* and fifteen putative ‘hybrids’, including fish collected beyond (MS28, MS31, MS32, MS35, MS42, MS46, MS51, and MS57) and within the *salmoides-floridanus* intergrade zone (Fig. 2). Clade II comprised 17 *M. salmoides* haplotypes plus polyphyletic *M. coosae* (one) and *M. punctulatus* (two). Notably,

within clade II, a haplotype from Cuatro Cienegas, México (MS70) fell out to a subclade (PP = 1) including haplotypes from Texas (Neuces and Guadalupe rivers) and New Mexico (Pecos River). Clades III (seven samples) and IV (four samples) formed a monophyletic group with two components: *Micropterus punctulatus* from the Gulf Coastal Plain (GCP) rivers west of Mobile Bay (Fig. 2) in clade III, and *Micropterus treculii* plus *M. punctulatus* haplotypes in coastal rivers directly east of Mobile Bay in clade IV. Clade V comprised all four *Micropterus notius* haplotypes. Clade VI (PP/BP = 1/95) was a mixture of 15 *M. coosae* and 18 *Micropterus henshalli* haplotypes. However, unresolved subclades VIa and VIb revealed non-allopatric intraspecific *M. coosae* structure in the Mobile Bay drainage. Clade VII (nine haplotypes) had three paraphyletic subclades: *M. coosae* haplotypes from the Broad and Savannah rivers (clade VIIc; PP/BP = 1/100) in a polytomy with two clades of *Micropterus cataractae* haplotypes (VII-a and VIIb; PP/BP = 0.99/96 and 1/100, respectively) from the drainages of the Chattahoochee River and Flint River systems. Clade VIII comprised all 17 *Micropterus dolomieu* plus 27 *M. punctulatus* and four *M. treculii* haplotypes. Clades I–V were always recovered as a monophyletic group with moderate support (PP/BP = 0.96/68) and relationships of the form (((clade I, clade II) (clade III, clade IV)), clade V). Clades III and IV were sister lineages (PP/BP = 1/99), and a sister relationship between mostly monophyletic *M. salmoides* and *M. s. floridanus* (clades I and II) was strongly supported (PP/BP = 1/88). The clade branching order was only partially resolved, with low

**Table 2.** Pairwise sequence divergence among *Micropterus* clades recovered during phylogenetic analyses in this paper

Clade	I	II	III	IV	V	VI	VIa	VIb	VII	VIIa	VIIb	VIIc	VIII	VIIIa	VIIIb
I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
II	0.040	–	–	–	–	–	–	–	–	–	–	–	–	–	–
III	0.076	0.080	–	–	–	–	–	–	–	–	–	–	–	–	–
IV	0.060	0.066	0.034	–	–	–	–	–	–	–	–	–	–	–	–
V	0.092	0.093	0.091	0.088	–	–	–	–	–	–	–	–	–	–	–
VI	0.107	0.113	0.102	0.090	0.114	–	–	–	–	–	–	–	–	–	–
VIa	0.112	0.115	0.106	0.095	0.117	–	–	–	–	–	–	–	–	–	–
VIb	0.116	0.121	0.105	0.089	0.117	–	0.025	–	–	–	–	–	–	–	–
VII	0.095	0.101	0.099	0.093	0.114	0.106	0.107	0.113	–	–	–	–	–	–	–
VIIa	0.093	0.099	0.097	0.092	0.108	0.106	0.106	0.113	–	–	–	–	–	–	–
VIIb	0.091	0.105	0.098	0.089	0.119	0.103	0.107	0.111	–	0.023	–	–	–	–	–
VIIc	0.100	0.103	0.101	0.099	0.117	0.107	0.108	0.116	–	0.026	0.027	–	–	–	–
VIII	0.128	0.129	0.119	0.125	0.143	0.132	0.135	0.140	0.113	0.115	0.116	0.108	–	–	–
VIIIa	0.131	0.130	0.120	0.129	0.144	0.133	0.136	0.142	0.115	0.117	0.119	0.110	–	–	–
VIIIb	0.126	0.128	0.117	0.122	0.142	0.132	0.136	0.139	0.110	0.112	0.113	0.106	–	0.012	–

Distances between well-supported major clades and their subclades (e.g. VIa) were corrected using the TIM3 + I +  $\Gamma$  DNA substitution model in PAUP\*.



**Figure 2.** Native geographical range distributions of *Micropterus* (Lee *et al.*, 1980; Boschung & Mayden, 2004) with approximate sampling localities superimposed. Numbers correspond to map numbers linked to haplotypes (Appendix); unnumbered sites have incomplete locality–haplotype information. The position of the putative *Micropterus salmoides* × *Micropterus salmoides floridanus* intergrade zone (top left; Bailey & Hubbs, 1949) and Mobile Bay (top right) are mapped. Scale bars: 300 km.

support values attributed to clades VII and VIII (Fig. 3). In contrast to patterns in clade I + II, clade VI *M. coosae* and *M. henshalli* were never recovered as strongly supported monophyletic taxa. Arkansas River basin *Micropterus dolomieu velox* (MD05 and MD06) were not distinct, but were recovered nested within *dolomieu* subclade VIIIA.

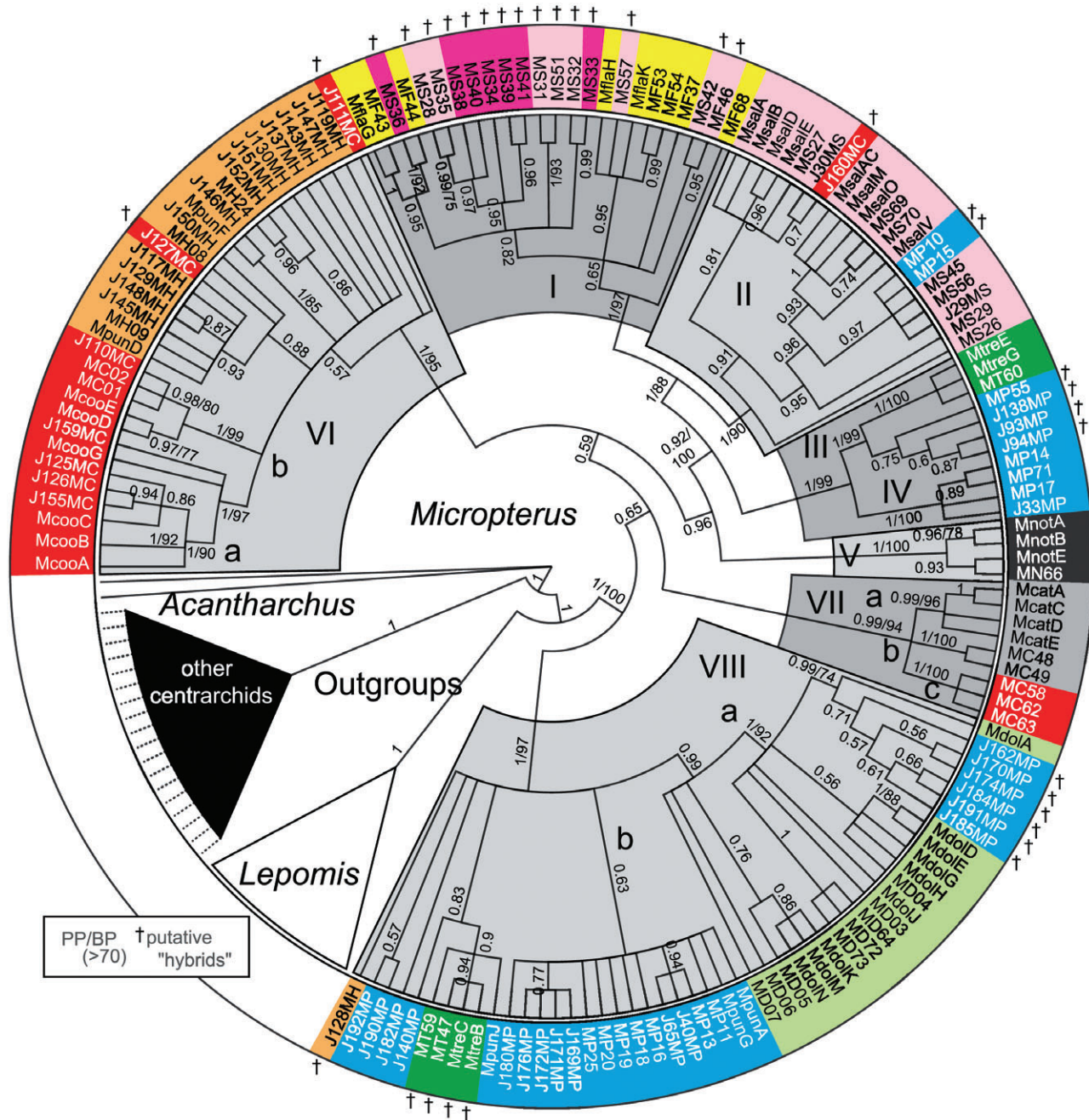
Haplotypes from fish identified as *M. salmoides* (clade I), *M. punctulatus* (clade II, subclade VIIIA, and clade III), *M. treculii* (clade VIIIB), and *M. coosae* (clades II, VI, and VII) generated eight forms of polyphyly representing putative hybridization-mediated mtDNA introgression events (Fig. 3). *Micropterus notius* haplotypes were never polyphyletic. *Micropterus cataractae* was only paraphyletic because of the clade VII polytomy.

## DISCUSSION

Through increased numerical and spatial sampling, we were able to recover a novel and complex pattern of

polyphyly among *Micropterus* species. Nonetheless, several general patterns agreeing with previous studies are evident. We have not obtained better nodal support for hypothesized relationships. *Micropterus* monophyly agrees with morphological (Branson & Moore, 1962; Chang, 1988; Wainwright & Lauder, 1992; Mabee, 1993) and molecular (Kassler *et al.*, 2002; Roe *et al.*, 2002; Near *et al.*, 2003, 2004, 2005) analyses sampling a range of characters. Our phylogeny also corroborates a close association between clades I–V, for example see Kassler *et al.* (2002). Large pairwise sequence divergences with *M. notius* (8.8–14.4%) and coastal *M. punctulatus* ( $\leq 9.9\%$ ; clade IV) stand out and correlate with strong support. The genetic and geographical distinctiveness of these lineages indicates a relatively higher degree of historical–contemporary isolation. Large sequence divergences ( $> 10\%$ ) in *M. dolomieu* comparisons seemingly reflect geographical isolation from other lineages, as *M. dolomieu* only overlaps notably with *M. salmoides* and *M. punctulatus* in its native range (Lee *et al.*, 1980).





**Figure 3.** Phylogeny of recognized *Micropterus* taxa inferred in MRBAYES from cytochrome *b* data. Major clades (in Roman numerals; a–c, subclades) are: I, *floridanus* clade; II, *salmoides* clade; III + IV, coastal clades; V, *notius* clade; VI, *coosae* clade; VII, *cataractae* clade; and VIII, *dolomieu* clade. Branches are cladogram-transformed, and are not proportional to lengths. Numbers  $\leq 1$  indicate Bayesian posterior probabilities; numbers  $> 70$  are maximum likelihood bootstrap proportions (ingroup only) along nodes. The colours along branch tips indicate the taxonomic/geographical origins of haplotypes, and correspond to colours used in Figure 2. Crosses indicate putative hybridization-mediated introgressions. Clades VI–VIII caused marginal internal node resolution and topological incongruencies across multiple runs (not shown). Haplotype codes correspond to the Appendix.

## MICROPTERUS POLYPHYLY, LINEAGE SORTING, AND HYBRIDIZATION-MEDIATED INTROGRESSION

By using restricted intraspecific sampling, previous molecular systematic analyses of *Micropterus* (e.g. Near *et al.*, 2003) operated on the assumption that taxa had monophyletic mtDNA and nuclear genomes. By contrast, the most outstanding result of our study is that we discovered eight forms of polyphyly *s.l.* This finding contributes to a growing body of evidence showing that the assumption of character monophyly is often invalid for animal taxa (Funk & Omland, 2003), and it begs further explanation. Many potential explanations exist for broad-sense polyphyly (Avice *et al.*, 1983; Avice, 1994; Funk & Omland, 2003). Unrecognized paralogy was ruled out because indicators of nuclear pseudogene sequences – indels, frame-shift mutations, etc. – were not present in our alignment. Given the non-adaptive nature of the *Micropterus* radiation, and hence close resemblance among species, polyphyly could have stemmed from morphological misdiagnosis. This seemed unlikely (particularly in clade VIII) because we diagnosed species using accepted standards (genetics and geographical variation in morphology; Boschung & Mayden, 2004), and we checked available voucher materials (especially for *M. punctulatus* and *M. henshalli*) to confirm identifications. Instead, polyphyly seemed likely to have arisen from mismatches between taxonomy and evolutionary diversity, or the impact of population genetic processes. We hypothesized three factors were responsible for broad-sense polyphyly.

First, and perhaps least important, an inadequate phylogenetic signal represents a potential explanation. A larger number of characters for such a large number of tips is more expensive and difficult to obtain, yet had such data been available to us it may have resolved some polyphyly, e.g. clades VI–VIII. A second possible explanation is incomplete lineage sorting (ILS). As mtDNA is haploid and uniparentally inherited, alleles coalesce four times faster than nDNA alleles (Moore, 1995). This results in a higher probability of recovering monophyly using mtDNA. Yet ILS may still occur when the time between lineage divergences is short (e.g. in the case of recent isolating barriers; Funk & Omland, 2003) or when morphological, geographical, or ecological divergence precedes genetic isolation. It seems ILS is unlikely to explain polyphyly among *Micropterus* lineages with shared haplotypes, yet moderate–deep divergences, e.g. clades I + II and III versus VIII. However, ILS seems highly plausible to explain para/polyphyly in more shallowly diverged lineages, e.g. clades III, VI, and VIII.

It is difficult to reject the possibility of some ILS. However, we favour the hypothesis that polyphyly is

best explained here by a third factor: historical or recent hybridization-mediated mtDNA introgression between *Micropterus* lineages. This is supported by independent evidence that *Micropterus* hybridization is fairly common. For example, genetic and morphological records show hybrids can attain high (> 20%) frequencies in *Micropterus* populations (Bolnick, 2009). This hypothesis also fits the view that post-mating gametic isolation is not an important reproductive barrier between centrarchid taxa (Bolnick, 2009). Moreover, evidence for *M. treculii* × *M. punctulatus* (or *M. dolomieu*) hybridization (clade III), *M. henshalli* × *M. coosae* (clade VI) hybridization, and *M. dolomieu* × *M. punctulatus* hybridization (subclade VIIa) in this study is consistent with genetic data from reservoirs in Missouri, Alabama, Georgia, South Carolina, and North Carolina (Koppelman, 1994; Pierce & Van Den Avyle, 1997; Avice *et al.*, 1997; Kassler *et al.*, 2002; Barwick *et al.*, 2006). To our knowledge, we found the first mtDNA-based evidence for putative *M. punctulatus* × *M. henshalli* (clade VIII) hybridizations. In future molecular investigations, it would be worthwhile to test this new evidence for hybridization using nuclear markers, e.g. nDNA support might rule out ILS. Doing so would also be relevant to elucidating the roles of ILS versus hybridization in the evolution of clade VI. *Micropterus henshalli* appears ecomorphologically differentiated (Gilbert, 1973; Baker, Johnston & Folkerts, 2008). Yet current data cannot distinguish whether *M. henshalli* is: (1) hybrid (*M. coosae* × *M. punctulatus*) in origin; (2) non-reciprocally monophyletic with *M. coosae* because of natural hybridization; or (3) a lineage in which the actual distinctness from *M. coosae* is obscured by ILS. Interestingly, there appear to be taxonomic and regional biases in *Micropterus* hybridization, with no evidence for introgression in some taxa, i.e. *M. notius*, and more putative hybrid sequences from southern (south of Tennessee; 29/35, 83%) versus northern (6/35, 17%) regions.

Our data support the hypothesis that broad-sense *Micropterus* polyphyly is attributable to natural hybridizations and hybridizations affected by anthropogenic translocations, i.e. stocking, of non-indigenous bass to 'improve' native fisheries (Pipas & Bulow, 1998; Philipp *et al.*, 2002). Mitochondrial DNA sites from one species enter the gene pool of another as completely linked pieces during introgression, through backcrossing of hybrids into parental stocks (Funk & Omland, 2003). An individual with one parental species phenotype carrying an mtDNA haplotype donated from another species indicates hybridization. Thus translocations are easily identifiable when suspected hybrid individuals fall out in clades of disjunct species, or when stocking records are avail-



able. However, if stocking records are not available and potentially hybridizing lineages have sympatric populations, then parental phenotypes in multiple mtDNA clades may represent natural hybridizations. We observed close proximity between collections of putative *M. dolomieu* × *M. punctulatus* and *M. treculii* × *M. punctulatus* ‘hybrids’, and other clade III and VIII localities. These patterns support natural hybridizations between these species. By contrast, the many putative *M. salmoides* × *M. s. floridanus* ‘hybrids’, collected between 70 and 700 km or further from other clade-I samples, indicate anthropogenic causes, namely management-related introgressive hybridization. This seems a particularly likely cause of haplotype MS57 polyphyly relative to other *M. salmoides*. This haplotype is from Vernon Lake, LA, a ‘trophy, quality, or special regulation lake’ stocked with *M. s. floridanus* since 1991 (B. Reed, Louisiana Department of Wildlife & Fisheries, pers. comm.). Hybridization between native and non-indigenous *Micropterus* as a result of anthropogenic translocations, e.g. at Vernon Lake, can erode local fish biodiversity. This is increasingly recognized. For example, introduced bass often swamp out genetic variation of native stocks through intraspecific and intrageneric introgression (Barwick *et al.*, 2006; also see discussion in Bolnick, 2009). Further studies are needed to evaluate introgressive *M. salmoides* × *M. s. floridanus* hybridization in stocked or presumably stocked bass populations (sampled here, and by others) to better gauge and manage potential damage to native population genetic diversity.

#### NOVEL *MICROPTERUS* BIODIVERSITY AND FUTURE STUDIES OF SPECIES LIMITS AND TAXONOMY

In addition to highlighting introgressive hybridization, our study has also revealed several novel biodiversity patterns. Clade VIIc *M. coosae* haplotypes from the Broad and Savannah rivers are notably distinct from other *M. coosae* haplotypes by 2.6–11.7% sequence divergence. These populations may represent an undescribed species, ‘Bartram’s Bass’ *Micropterus* sp. cf. *coosae* (B. Freeman, pers. comm.). This study provides a starting point for adding a genetic perspective to the current work describing this taxon (B. Freeman, pers. comm.). Our finding that coastal (clade IV) *M. punctulatus* populations are monophyletic and genetically distinct (3.4–12.9% sequence divergence) is also unique. If this indicates cryptic, species-level diversity, then paraphyletic clade-III *M. punctulatus* could reflect a secondary contact zone between clade-IV *M. punctulatus* and *M. treculii* west of Mobile Bay (Fig. 2). We realize the limitations of our approach based on mtDNA alone.

Therefore, we have avoided drawing detailed taxonomic conclusions from our results, which would be premature. However, these novel biodiversity patterns deserve more attention. Our mtDNA data should be integrated with unlinked nuclear loci and morphological data in future analyses delimiting species boundaries and evaluating *Micropterus* taxonomy. Our data should provide a useful taxon-sampling guide for expanding character sampling in these and more in-depth phylogeography studies, which should continue to yield insights into the history, distinctiveness, and geographical distributions of genetic lineages within and among *Micropterus*.

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## APPENDIX

Specimen numbers, locality information, collection numbers, and GenBank accession numbers. Whether sequences came from this or another study is indicated with the reference citation. Samples are from the USA unless stated otherwise. Map numbers correspond to locality labels in Figure 2 and haplotype codes correspond to Figure 3. 'Species' correspond to initial identifications.

Species (haplotype code)	ST	County	Locality	Map no.	Collection no.	Reference	GenBank no.
<i>Micropterus</i> ingroup							
<i>M. cataractae</i> (MC48)	GA	Heard	Hillabahatchee Creek	1	DAN01.44	This study	HM070885
<i>M. cataractae</i> (MC49)	GA	Heard	Hillabahatchee Creek	1	DAN01.44	This study	HM070886
<i>M. coosae</i> (MC01)	AL	Blount	Blue Springs Creek	2	UAIC 12471.05	This study	HM070843
<i>M. coosae</i> (MC02)	AL	Blount	Gurley Creek	3	JCB0719	This study	HM070844
<i>M. coosae</i> (MC58)	GA	Madison	South Fork Broad River at Ogelthorpe	4	SLUM 379.07	This study	HM070892
<i>M. coosae</i> (MC62)	GA	Madison	South Fork Broad River at Ogelthorpe	4	SLUM 379.07	This study	HM070895
<i>M. coosae</i> (MC63)	GA	–	Savannah River	5	UAIC 12376.01	This study	HM070896
<i>M. coosae</i> (J155MC)	AL	Etowah	Big Will's Creek at US Highway 11 at Attalla	6	JCB0742	This study	HM070942
<i>M. coosae</i> (J159MC)	AL	Etowah	Big Will's Creek at US Highway 11 at Attalla	6	JCB0742	This study	HM070943
<i>M. coosae</i> (J160MC)	AL	St. Clair	Little Canoe Creek at US Highway 11	7	JCB0741	This study	HM070944
<i>M. coosae</i> (J125MC)	AL	DeKalb	Big Will's Creek at Reed City	8	JCB0726	This study	HM070945
<i>M. coosae</i> (J126MC)	AL	DeKalb	Big Will's Creek at Collinsville	9	JCB0727	This study	HM070946
<i>M. coosae</i> (J127MC)	AL	DeKalb	Big Will's Creek at Collinsville	9	JCB0727	This study	HM070947
<i>M. coosae</i> (J111MC)	AL	Blount	Gurley Creek	3	JCB0719	This study	HM070948
<i>M. coosae</i> (J110MC)	AL	Blount	Gurley Creek	3	JCB0719	This study	HM070949
<i>M. dolomieu</i> (MD03)	AL	Colbert	Bear Creek, 1.4 miles east of Bishop	10	UAIC 12319.19	This study	HM070845
<i>M. dolomieu</i> (MD04)	OH	Ottawa	Lake Erie at OH State Route 53	11	UAIC 12504.01	This study	HM070846
<i>M. dolomieu</i> (MD05)	AK	Washington	Illinois River, 5 km south of Robinson	12	UAIC 12550.18	This study	HM070847
<i>M. dolomieu</i> (MD06)	AK	Montgomery	Ouachita River at US Highway 270	13	UAIC 12681.17	This study	HM070848
<i>M. dolomieu</i> (MD07)	TX	Val Verde	Devil's River 12 miles west of Loma Alta	14	UAIC 13508.09	This study	HM070849
<i>M. dolomieu</i> (MD64)	NM	–	Pecos River	15	SLUM 657.02	This study	HM070897
<i>M. dolomieu</i> (MD72)	KY	–	Green River	16	SLUM 625.05	This study	HM070903
<i>M. dolomieu</i> (MD73)	MO	–	North Fork White River	17	SLUM 757.03	This study	HM070904
<i>M. henshalli</i> (MH08)	AL	Bibb	Cahaba River in Centreville	18	UAIC 11310.11	This study	HM070850
<i>M. henshalli</i> (MH09)	AL	Talladega	Averett Branch, 0.8 miles east of Fayetteville	19	UAIC 12259.02	This study	HM070851
<i>M. henshalli</i> (MH24)	AL	Bibb	Cahaba River at US Highway 82 in Centreville	20	UAIC 12715.16	This study	HM070862
<i>M. henshalli</i> (J151MH)	AL	Macon	Uphapee Creek at US Highway 80, east of Tuskegee	21	JCB0747	This study	HM070914
<i>M. henshalli</i> (J145MH)	AL	Macon	Uphapee Creek at AL State Route 49, south of Tallassee	22	JCB0746	This study	HM070929
<i>M. henshalli</i> (J148MH)	AL	Macon	Uphapee Creek at US Highway 80, east of Tuskegee	21	JCB0747	This study	HM070930
<i>M. henshalli</i> (J150MH)	AL	Macon	Uphapee Creek at US Highway 80, east of Tuskegee	21	JCB0747	This study	HM070931
<i>M. henshalli</i> (J152MH)	AL	Macon	Uphapee Creek at US Highway 80, east of Tuskegee	21	JCB0747	This study	HM070932
<i>M. henshalli</i> (J130MH)	AL	Shelby	Cahaba River at County Road 52, near Helena	23	JCB0730	This study	HM070933
<i>M. henshalli</i> (J128MH)	AL	Calhoun	Choccolocco Creek at AL State Route 21, in Oxford	24	JCB0725	This study	HM070934
<i>M. henshalli</i> (J129MH)	AL	Calhoun	Choccolocco Creek at AL State Route 21, in Oxford	24	JCB0725	This study	HM070935

APPENDIX *Continued*

Species (haplotype code)	ST	County	Locality	Map no.	Collection no.	Reference	GenBank no.
<i>M. henshalli</i> (J117MH)	AL	Jefferson	Fivemile Creek at AL State Route 5	25	JCB0724	This study	HM070936
<i>M. henshalli</i> (J119MH)	AL	Jefferson	Fivemile Creek at AL State Route 5	25	JCB0724	This study	HM070937
<i>M. henshalli</i> (J137MH)	AL	Calhoun	Big Flat Creek	26	JCB06101	This study	HM070938
<i>M. henshalli</i> (J143MH)	AL	Chilton	Little Mulberry Creek at AL State Route 22	27	JCB0743	This study	HM070939
<i>M. henshalli</i> (J146MH)	AL	Elmore	Mortar Creek at AL State Route 14 at Coosada	28	JCB0745	This study	HM070940
<i>M. henshalli</i> (J147MH)	AL	Elmore	Mortar Creek at AL State Route 14 at Coosada	28	JCB0745	This study	HM070941
<i>M. notius</i> (MN66)	FL	—	Santa Fe River	29	UAIC 12291.02	This study	HM070898
<i>M. punctulatus</i> (MP10)	GA	Harris	Mountain Creek	30	UAIC12370.04	This study	HM070852
<i>M. punctulatus</i> (MP11)	KY	Rockcastle	Horse Lick Creek, 10 km NE of Livingston	31	UAIC 12460.08	This study	HM070853
<i>M. punctulatus</i> (MP13)	AL	Lauderdale	Shoal Creek, 3 miles SE of Pruittton	32	UAIC 12509.15	This study	HM070854
<i>M. punctulatus</i> (MP14)	AL	Escambia	Little Escambia Creek at US Highway 29/31	33	UAIC 12548.16	This study	HM070855
<i>M. punctulatus</i> (MP15)	AK	Benton	Elk River at AR Highway 59	34	UAIC 12549.14	This study	HM070856
<i>M. punctulatus</i> (MP16)	AK	Washington	Tributary to Neosho River	35	UAIC 12550.19	This study	HM070857
<i>M. punctulatus</i> (MP17)	FL	Okaloosa	Pond Creek at County Road 393, 3 km north of Dorcas	36	UAIC 12594.14	This study	HM070858
<i>M. punctulatus</i> (MP18)	AK	Benton	War Eagle Creek at AR Highway 303	37	UAIC 12596.2	This study	HM070859
<i>M. punctulatus</i> (MP19)	AK	Scott	Poteau River at AR Highway 80 SE of Hon	38	UAIC 12598.13	This study	HM070860
<i>M. punctulatus</i> (MP20)	FL	Holmes	Ponce de Leon Spring	39	UAIC 12649.11	This study	HM070861
<i>M. punctulatus</i> (MP25)	AK	Calhoun	Lost Creek boat ramp	40	UAIC 12754.15	This study	HM070863
<i>M. punctulatus</i> (MP55)	MS	Amite	Beaver Creek at MS Highway 560	41	UAIC 13776.02	This study	HM070890
<i>M. punctulatus</i> (MP71)	AL	—	Conceh River	42	UAIC 13418	This study	HM070902
<i>M. punctulatus</i> (J138MP)	LA	Tangipahoa	Tangipahoa River at I-12	43	JCB0739B	This study	HM070905
<i>M. punctulatus</i> (J93MP)	MS	Marion	Clear Creek	44	JCB0713	This study	HM070906
<i>M. punctulatus</i> (J94MP)	MS	Pearl River	West Hobolochitto Creek	45	JCB0714	This study	HM070907
<i>M. punctulatus</i> (J33MP)	AL	Lauderdale	Sixmile Creek	46	GSA2564	This study	HM070908
<i>M. punctulatus</i> (J140MP)	TX	—	Trinity River	47	JCB0748	This study	HM070909
<i>M. punctulatus</i> (J40MP)	MS	Tishomingo	Bear Creek	48	GSA38899	This study	HM070912
<i>M. punctulatus</i> (J65MP)	MS	Tishomingo	Bear Creek at MS State Route 86	49	JCB0650	This study	HM070913
<i>M. punctulatus</i> (J162MP)	OH	—	Ohio River, mile 648.7	50	JCB0749	This study	HM070915
<i>M. punctulatus</i> (J169MP)	OH	—	Ohio River, mile 731.5	51	JCB0752	This study	HM070916
<i>M. punctulatus</i> (J171MP)	OH	—	Ohio River, mile 55.6	52	JCB0756	This study	HM070917
<i>M. punctulatus</i> (J172MP)	OH	—	Ohio River, mile 58.1	53	JCB0757	This study	HM070918
<i>M. punctulatus</i> (J170MP)	OH	—	Ohio River, mile 380.4	54	JCB0755	This study	HM070919
<i>M. punctulatus</i> (J174MP)	OH	—	Ohio River, mile 384.8	55	JCB0758	This study	HM070920
<i>M. punctulatus</i> (J176MP)	OH	—	Ohio River, mile 58.1	56	JCB0757	This study	HM070921
<i>M. punctulatus</i> (J180MP)	OH	—	Ohio River, mile 599.0	57	JCB0768	This study	HM070922
<i>M. punctulatus</i> (J182MP)	OH	—	Ohio River, mile 62.8	58	JCB0760	This study	HM070923
<i>M. punctulatus</i> (J184MP)	OH	—	Ohio River, mile 430.0	59	JCB0762	This study	HM070924
<i>M. punctulatus</i> (J185MP)	OH	—	Ohio River, mile 430.0	60	JCB0762	This study	HM070925
<i>M. punctulatus</i> (J190MP)	OH	—	Ohio River, mile 395.0	61	JCB0763	This study	HM070926

<i>M. punctulatus</i> (J191MP)	OH	–	Ohio River, mile 430.0	62	JCB0762	This study	HM070927
<i>M. punctulatus</i> (J192MP)	OH	–	Ohio River, mile 79.3	63	JCB0765	This study	HM070928
<i>M. s. floridanus</i> (MF37)	FL	Columbia	Santa Fe River at US Highway 41/441	69	UAIC 12603.11	This study	HM070874
<i>M. s. floridanus</i> (MF43)	FL	Lake	Alexander Spring at FL Highway 445	74	UAIC 12720.14	This study	HM070880
<i>M. s. floridanus</i> (MF44)	FL	Hillsborough	Lithia Spring, ESE of Riverview	75	UAIC 12727.11	This study	HM070881
<i>M. s. floridanus</i> (MF53)	FL	Dade	Everglades	78	UAIC 12368.01	This study	HM070888
<i>M. s. floridanus</i> (MF54)	FL	Dade	Everglades	78	UAIC 12368.01	This study	HM070889
<i>M. s. floridanus</i> (MF68)	FL	–	St. John's River	79	UAIC 12290.05	This study	HM070899
<i>M. salmoides</i> (MS26)	AL	Jefferson	Turkey Creek	80	UAIC 11712.01	This study	HM070864
<i>M. salmoides</i> (MS27)	MD	Charles	Zekiah Swamp Run at MD Highway 5	81	UAIC 12263.02	This study	HM070865
<i>M. salmoides</i> (MS28)	AL	Escambia	Little Escambia Creek at US Highway 29/31	33	UAIC 12548.17	This study	HM070866
<i>M. salmoides</i> (MS29)	NC	Nash	Tar River 2 km south of West Mount	82	UAIC 12586.04	This study	HM070867
<i>M. salmoides</i> (MS31)	NC	Robeson	Black Swamp, 12 km WSW of Lumberton	64	UAIC 12589.13	This study	HM070868
<i>M. salmoides</i> (MS32)	SC	Clarendon	Jack's Creek at SC Highway 26	65	UAIC 12590.09	This study	HM070869
<i>M. salmoides</i> (MS33)	SC	Jasper	Tributary of Savannah River, 1 km south of Purysburgh	66	UAIC 12591.03	This study	HM070870
<i>M. salmoides</i> (MS34)	FL	Okaloosa	Pond Creek at County Road 393, 3 km north of Dorcas	36	UAIC 12594.15	This study	HM070871
<i>M. salmoides</i> (MS35)	AL	Pike	Indian Creek, 14 km west of Troy	67	UAIC 12597.12	This study	HM070872
<i>M. salmoides</i> (MS36)	FL	Marion	Orange Creek at FL Highway 315	68	UAIC 12601.06	This study	HM070873
<i>M. salmoides</i> (MS38)	FL	Holmes	Ponce de Leon Spring	39	UAIC 12649.12	This study	HM070875
<i>M. salmoides</i> (MS39)	FL	Gadsden	Ochlockonee River	70	UAIC 12650.15	This study	HM070876
<i>M. salmoides</i> (MS40)	FL	Franklin	Graham Creek at FL Highway 65	71	UAIC 12651.14	This study	HM070877
<i>M. salmoides</i> (MS41)	FL	Johnson	Spring Creek at US Highway 90	72	UAIC 12684.10	This study	HM070878
<i>M. salmoides</i> (MS42)	NC	Johnston	Little River at Lowell Mill, off County Road 2335	73	UAIC 12699.16	This study	HM070879
<i>M. salmoides</i> (MS45)	AK	Calhoun	Lost Bayou	83	UAIC 12754.16	This study	HM070882
<i>M. salmoides</i> (MS46)	NC	Nash	Sapony Creek, SSE of Nashville	76	UAIC 13069.09	This study	HM070883
<i>M. salmoides</i> (MS51)	NC	Columbus	Lake Waccamaw	77	UAIC 12588.09	This study	HM070887
<i>M. salmoides</i> (MS56)	LA	Ouachita	Bayou Dofiard, ULM campus	84	UAIC 13780.02	This study	HM070891
<i>M. salmoides</i> (MS57)	LA	Vernon	Vernon Lake	84b	UAIC 13775.03	This study	–
<i>M. salmoides</i> (MS69)	NM	–	Pecos River	15	SLUM 657.03	This study	HM070900
<i>M. salmoides</i> (MS70)	–	–	Cuatro Cienegas, Coahuila, Mexico	85	RLM00.42	This study	HM070901
<i>M. salmoides</i> (J29MS)	AL	Lauderdale	Sixmile Creek	86	JCB0662	This study	HM070910
<i>M. salmoides</i> (J30MS)	AL	Lauderdale	Sixmile Creek	86	JCB0662	This study	HM070911
<i>M. treculii</i> (MT47)	TX	Kimble	South Llano River at TX County Road 2169	87	UAIC 13512.08	This study	HM070884
<i>M. treculii</i> (MT59)	TX	Kendall	Guadalupe River	88	UAIC 13506.08	This study	HM070893
<i>M. treculii</i> (MT60)	TX	Kendall	Guadalupe River	88	UAIC 13506.08	This study	HM070894
<i>M. cataractae</i> (MeatA)	AL	Russell	Little Uchee Creek	89	–	Near <i>et al.</i> (2003)	AY225714
<i>M. cataractae</i> (MeatC)	AL	Russell	Little Uchee Creek	89	–	Near <i>et al.</i> (2003)	AY225715
<i>M. cataractae</i> (MeatD)	GA	Crisp	Flint River	90	–	Near <i>et al.</i> (2003)	AY225716
<i>M. cataractae</i> (MeatE)	GA	Crisp	Flint River	90	–	Near <i>et al.</i> (2003)	AY225717



APPENDIX *Continued*

Species (haplotype code)	ST	County	Locality	Map no.	Collection no.	Reference	GenBank no.
<i>M. coosae</i> (McooA)	AL	Cherokee	Terrapin Creek, Coosa River	91	–	Near <i>et al.</i> (2003)	AY225708
<i>M. coosae</i> (McooB)	AL	Cherokee	Terrapin Creek, Coosa River	91	–	Near <i>et al.</i> (2003)	AY225709
<i>M. coosae</i> (McooC)	AL	Cherokee	Terrapin Creek, Coosa River	91	–	Near <i>et al.</i> (2003)	AY225710
<i>M. coosae</i> (McooD)	AL	Chambers	Tallapoosa River	92	–	Near <i>et al.</i> (2003)	AY225711
<i>M. coosae</i> (McooE)	AL	Chambers	Tallapoosa River	92	–	Near <i>et al.</i> (2003)	AY225712
<i>M. coosae</i> (McooG)	TN	Polk	Conasauga River	93	INHS 41809, TJN 440	Near <i>et al.</i> (2004)	AY225713
<i>M. dolomieu</i> (MdolA)	–	–	‘Virginia’	–	–	Near <i>et al.</i> (2003)	AY225685
<i>M. dolomieu</i> (MdolD)	WI	Kenosha	Fox River	94	–	Near <i>et al.</i> (2003)	AY225687
<i>M. dolomieu</i> (MdolE)	WI	Kenosha	Fox River	94	–	Near <i>et al.</i> (2003)	AY225688
<i>M. dolomieu</i> (MdolG)	–	–	‘Miller’s’	–	–	Near <i>et al.</i> (2003)	AY225689
<i>M. dolomieu</i> (MdolH)	–	–	‘Miller’s’	–	–	Near <i>et al.</i> (2003)	AY225690
<i>M. dolomieu</i> (MdolJ)	–	–	‘Guadalupe’	–	–	Near <i>et al.</i> (2003)	AY225691
<i>M. dolomieu</i> (MdolK)	MO	MacDonald	Big Sugar Creek	95	–	Near <i>et al.</i> (2003)	AY225692
<i>M. dolomieu</i> (MdolM)	MO	–	Shoal Creek	96	–	Near <i>et al.</i> (2003)	AY225693
<i>M. dolomieu</i> (MdolN)	MO	–	Shoal Creek	96	–	Near <i>et al.</i> (2003)	AY225694
<i>M. s. floridanus</i> (MflaG)	FL	Lake	Lake Eustis	97	–	Near <i>et al.</i> (2003)	AY225669
<i>M. s. floridanus</i> (MflaH)	FL	Lake	Lake Eustis	97	–	Near <i>et al.</i> (2003)	AY225670
<i>M. s. floridanus</i> (MflaJ)	FL	–	Lake Istokpoga	98	–	Near <i>et al.</i> (2003)	AY225672
<i>M. s. floridanus</i> (MflaK)	FL	–	Lake Istokpoga	98	–	Near <i>et al.</i> (2003)	AY225673
<i>M. notius</i> (MnotA)	FL	Jefferson	Wacissa River	99	–	Near <i>et al.</i> (2003)	AY225704

<i>M. notius</i> (MnotB)	FL	Jefferson	Wacissa River	99	–	Near <i>et al.</i> (2003)	AY225705
<i>M. notius</i> (MnotE)	FL	Alachua	Santa Fe River	100	–	Near <i>et al.</i> (2003)	AY225707
<i>M. punctulatus</i> (MpunA)	KA	Chase	Chase Lake	101	–	Near <i>et al.</i> (2003)	AY225695
<i>M. punctulatus</i> (MpunD)	–	–	–	–	–	Near <i>et al.</i> (2003)	–
<i>M. punctulatus</i> (MpunF)	–	–	–	–	–	Near <i>et al.</i> (2003)	–
<i>M. punctulatus</i> (MpunG)	TN	Union	Norris Reservoir, Clinch River	102	–	Near <i>et al.</i> (2003)	AY225698
<i>M. punctulatus</i> (MpunJ)	TX	Bosque	Cedron Creek	103	–	Near <i>et al.</i> (2003)	AY225700
<i>M. punctulatus</i> (MpunK)	TX	Hill	Lake Whitney	104	–	Near <i>et al.</i> (2003)	AY225761
<i>M. salmoides</i> (MsalA)	WI	Burnett	Lipset Lake	105	–	Near <i>et al.</i> (2003)	AY225675
<i>M. salmoides</i> (MsalAC)	TX	–	‘Guadalupe’	106	–	Near <i>et al.</i> (2003)	AY225684
<i>M. salmoides</i> (MsalB)	WI	Burnett	Lipset Lake	105	–	Near <i>et al.</i> (2003)	AY225676
<i>M. salmoides</i> (MsalD)	–	–	‘Long’	–	–	Near <i>et al.</i> (2003)	AY225677
<i>M. salmoides</i> (MsalE)	–	–	‘Long’	–	–	Near <i>et al.</i> (2003)	AY225678
<i>M. salmoides</i> (unknown)	IL	Moultrie	Lake Shelbyville	–	–	Near <i>et al.</i> (2003)	–
<i>M. salmoides</i> (MsalM)	TX	Uvalde	Neuces River	107	–	Near <i>et al.</i> (2003)	AY225679
<i>M. salmoides</i> (MsalO)	TX	Uvalde	Neuces River	107	–	Near <i>et al.</i> (2003)	AY225680
<i>M. salmoides</i> (MsalV)	–	–	Pedrenales River	108	–	Near <i>et al.</i> (2003)	AY225683
<i>M. treculii</i> (MtreB)	TX	–	Llano River	109	–	Near <i>et al.</i> (2003)	–
<i>M. treculii</i> (MtreC)	TX	–	Llano River	109	–	Near <i>et al.</i> (2003)	–
<i>M. treculii</i> (MtreE)	TX	Burnet	Lake Buchanan	110	–	Near <i>et al.</i> (2003)	AY225702
<i>M. treculii</i> (MtreG)	TX	Burnet	Lake Buchanan	110	–	Near <i>et al.</i> (2003)	AY225703
Outgroups							

APPENDIX *Continued*

Species (haplotype code)	ST	County	Locality	Map no.	Collection no.	Reference	GenBank no.
<i>Acantharchus pomotis</i>	—	—	—	—	UAIC 11844.02	Roe <i>et al.</i> (2002)	AY115994
<i>Acantharchus pomotis</i>	—	—	—	—	INHS 45049	Roe <i>et al.</i> (2002)	AY115993
<i>Ambloplites cavifrons</i>	—	—	—	—	NCSM 30358	Near & Benard (2004)	AY770839
<i>Ambloplites cavifrons</i>	—	—	—	—	UAIC 13074.02	Roe <i>et al.</i> (2002)	AY115980
<i>Ambloplites rupestris</i>	—	—	—	—	TJN 284	Near <i>et al.</i> (2003)	AY225663
<i>Ambloplites rupestris</i>	—	—	—	—	SIUC 37911	Roe <i>et al.</i> (2002)	AY115978
<i>Archoplites interruptus</i>	—	—	—	—	INHS 59069, TJN 1077	Near <i>et al.</i> (2003)	AY225665
<i>Archoplites interruptus</i>	—	—	—	—	—	Roe <i>et al.</i> (2002)	AY115996
<i>Centrarchus macropterus</i>	—	—	—	—	INHS 38384, TJN 384	Near <i>et al.</i> (2003)	AY225666
<i>Centrarchus macropterus</i>	—	—	—	—	UAIC 13070.07	Roe <i>et al.</i> (2002)	AY115982
<i>Enneacanthus chaetodon</i>	—	—	—	—	UAIC 1184.03	Roe <i>et al.</i> (2002)	AY115984
<i>Enneacanthus chaetodon</i>	—	—	—	—	UAIC 1313904.04	Roe <i>et al.</i> (2002)	AY115983
<i>Enneacanthus gloriosus</i>	—	—	—	—	UAIC 12367.01	Roe <i>et al.</i> (2002)	AY115986
<i>Enneacanthus gloriosus</i>	—	—	—	—	UAIC 11704.14	Roe <i>et al.</i> (2002)	AY115985
<i>Enneacanthus obesus</i>	—	—	—	—	INHS 38762, TJN 379	Near <i>et al.</i> (2003)	AY225664
<i>Enneacanthus obesus</i>	—	—	—	—	UAIC 11844.04	Roe <i>et al.</i> (2002)	AY115988
<i>Lepomis aurtitus</i>	—	—	—	—	UAIC 12591.01	Harris <i>et al.</i> (2005)	AY828957
<i>Lepomis cyanellus</i>	—	—	—	—	UAIC 12285.04	Harris <i>et al.</i> (2005)	AY828959



<i>Lepomis gulosus</i>	—	—	—	UAIC 11865.03	Harris <i>et al.</i> (2005)	AY828963
<i>Lepomis gibbosus</i>	—	—	—	UAIC 11834.01	Harris <i>et al.</i> (2005)	AY828962
<i>Lepomis humilis</i>	—	—	—	KU 25192	Harris <i>et al.</i> (2005)	AY828965
<i>Lepomis macrochirus</i>	—	—	—	UAIC 12511.07	Harris <i>et al.</i> (2005)	AY828968
<i>Lepomis marginatus</i>	—	—	—	UAIC 12290.03	Harris <i>et al.</i> (2005)	AY828969
<i>Lepomis megalotis</i>	—	—	—	UAIC 12354.03	Harris <i>et al.</i> (2005)	AY828975
<i>Lepomis microlophus</i>	—	—	—	UAIC 12650.11	Harris <i>et al.</i> (2005)	AY828982
<i>Lepomis miniatus</i>	—	—	—	UAIC 12679.05	Harris <i>et al.</i> (2005)	AY828990
<i>Lepomis punctatus</i>	—	—	—	UAIC 12291.01	Harris <i>et al.</i> (2005)	AY828994
<i>Lepomis symmetricus</i>	—	—	—	OSUS 27537	Harris <i>et al.</i> (2005)	AY828996
<i>Pomoxis annularis</i>	—	—	—	UAIC 12610.08	Roe <i>et al.</i> (2002)	AY115990
<i>Pomoxis annularis</i>	—	—	—	UAIC 11821.02	Roe <i>et al.</i> (2002)	AY115989
<i>Pomoxis nigromaculatus</i>	—	—	—	OSUS 27536	Roe <i>et al.</i> (2002)	AY115992
<i>Pomoxis nigromaculatus</i>	—	—	—	UAIC 12309.04	Roe <i>et al.</i> (2002)	AY115991

DAN, D. A. Neeley, personal collection; GSA, Geological Survey of Alabama Fish Collection; INHS, Illinois Natural History Survey; JCB, J. C. Bagley, personal collection; KU, University of Kansas; NCSM, North Carolina State Museum; OSUS, Oklahoma State University; RLM, R. L. Mayden, personal collection; SIUC, Southern Illinois University, Carbondale; SLUM, St. Louis University Museum; UAIC, University of Alabama Ichthyological Collection.