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Two new species of *Micropterus* (Centrarchidae) endemic to Atlantic Slope river drainages in Georgia, South Carolina and North Carolina, U.S.A.

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Abstract

We describe as new species *Micropterus pucpuggy* Freeman & Freeman (Bartram's Bass), **sp. nov.**, and *Micropterus calliurus* Freeman & Freeman (Altamaha Bass), **sp. nov.**, which occur allopatrically in four river systems draining the Atlantic Slope of the southeastern United States. In recent decades, biologists and anglers have acknowledged the existence of these two distinctive taxa of black bass, both of which were previously considered synonymous with *M. coosae* Hubbs & Bailey (Redeye Bass). However, introgression with non-native congeners that have been widely introduced for sport-fishing (including *M. henshalli* Hubbs & Bailey and *M. dolomieu* Lacepède) has confounded formal description of *M. pucpuggy* and *M.calliurus*. We examined mitochondrial (mtDNA) and nuclear gene sequences of candidate type-specimens of *M. pucpuggy* and *M. calliurus*. We then used reduced-representation, short-read sequencing of candidate types along with specimens of six other *Micropterus* species to identify a series of non-introgressed individuals for each of the two new species. *Micropterus pucpuggy* and *M. calliurus* are each reciprocally monophyletic in both mitochondrial and RADseq phylogenies and are diagnosable from all other *Micropterus* species and from each other in chromatic fin coloration, body pigmentation, and other morphological attributes.

Key words: Redeye Basses, *Micropterus coosae*, Black Basses, Altamaha Bass, Bartram's Bass

"All too often the introduction of exotic species has exterminated the local forms" (Hubbs & Bailey 1940)

Introduction

Black basses, genus *Micropterus* Lacepède, comprise a group of 14 currently described species in the family Centrarchidae, all of which are endemic to eastern North America, with the greatest diversity in the southern United States (US; Kim *et al.* 2022). Anglers have long prized the black basses as sport fishes (Henshall 1881; Long *et al.* 2015), and black bass are ecologically important predators in lakes, reservoirs and rivers (Power *et al.* 1985; Jackson 2002). More recently, conservationists and managers have come to appreciate the full taxonomic diversity of the black bass clade (Taylor *et al.* 2019). As recently as 2011, biologists formally recognized only eight species of *Micropterus*, however taxonomists working with this group have long noted the probable existence of unnamed,

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narrowly distributed taxa. In fact, in their 1940 revision of the black basses, Hubbs and Bailey pointed to the southern states as likely harboring localized and unnamed variants (Hubbs & Bailey 1940). Their observation was prescient in that 10 of the 14 described *Micropterus* species are geographically restricted to one or a few southern US river systems.

Recent taxonomic work has focused on lineage divergence within the Redeye Bass, *Micropterus coosae* Hubbs & Bailey, complex (Figure 1). As originally described, *M. coosae* occupied Gulf and Atlantic slope rivers extending from the Mobile River drainage in the west to the Savannah River in the east (Hubbs & Bailey 1940). Hubbs and Bailey based the *M. coosae* description primarily on individuals collected in the upper Mobile River drainage (i.e., 125 of 134 type specimens); the authors reported a single specimen taken from the Savannah River at Augusta Georgia (USNM 17112, 1877) along with eight specimens from streams in the Chattahoochee River system. Baker *et al.* (2013) recognized four new species (*M. warriorensis* Baker, Johnston & Blanton, *M. tallapoosae* Baker, Johnston & Blanton, *M. cahabae* Baker, Johnston & Blanton, and *M. chattahoochae* Baker, Johnston & Blanton) endemic to river systems within the Gulf slope portion of the presumed *M. coosae* range (Baker *et al.* 2013; Kim *et al.* 2022). Biologists and anglers have also recognized distinctive forms of *M. coosae* in the eastern Atlantic Slope portion of that species' range as delimited by Hubbs & Bailey (1940). In particular, the form that occurs in the Savannah River drainage has been informally referred to as Bartram's Bass for over 20 years (Bagley *et al.* 2011). Baker *et al.* (2013) identified this form and the form native to the Altamaha River drainage (informally, Altamaha Bass) as distinct from *M. coosae* but did not describe or delimit these species. Freeman *et al.* (2015) and Kim *et al.* (2022) subsequently reported the distinctiveness of Bartram's Bass and Altamaha Bass based on mitochondrial and nuclear genes, and genome-wide short-read sequences, respectively. Until now, however, Bartram's Bass and Altamaha Bass have lacked formal species recognition.

Introgression with non-native *Micropterus* species introduced into the native ranges of Bartram's Bass (Leitner *et al.* 2015; Bangs *et al.* 2018; Peoples *et al.* 2021) and Altamaha Bass has complicated formal species descriptions. Introgression of native with non-native black basses has occurred widely where non-native bass have been introduced to diversify sport-fisheries (Koppelman & Garrett 2002; Baker *et al.* 2013; Kim *et al.* 2022). The difficulty for species description is that hybridization can obscure traits that define a distinctive taxon, and visual inspection cannot reliably identify introgressed individuals (Dakin *et al.* 2015; Freeman *et al.* 2015).

Herein we formally describe Bartram's Bass and Altamaha Bass as new species based on individuals assessed as non-introgressed using reduced-representation, short-read sequencing. We provide species diagnoses, update the distributional ranges for both taxa, and discuss conservation challenges.

Materials and Methods

We analyzed specimens of the two new *Micropterus* species collected, respectively, at 14 localities in the Savannah and Saluda River basins and 14 localities in the Altamaha and Ogeechee River basins (Figure 1, Supplement 1) for evidence of introgression with non-native bass species (specifically *M. henshalli* and *M. dolomieu*). Specimens were collected by electrofishing, seining, and hook and line. Tissues (fin-clips or muscle tissue) were preserved in 95% ethanol or in salt-saturated dimethyl sulfoxide in the field, or in some cases, whole fish were preserved in 95% ethanol or frozen in the field and returned to the lab. Tissue-only samples were retained for a total of nine specimens of the two new species (See *Non-type Tissue Samples* and Supplement 1). All tissue samples utilized were accessioned into the Georgia Museum of Natural History Tissue Collection (GMNHTC). We also extracted genomic DNA from GMNHTC-archived tissue samples of six *Micropterus* species representing presumed closest relatives of the new taxa (i.e., *M. chattahoochae* n=4; *M. coosae* n=5; *M. cataractae* n=4), taxa introduced in the range of the new taxa (*M. henshalli* n= 4; *M. dolomieu* n=6), and *M. salmoides* (n=1, used as an outgroup in phylogenetic analysis; See *Non-type Tissue Samples*).

Genomic DNA was extracted from fin or muscle tissues using a modified Puregene method (Wares 2024) or by using a Qiagen DNeasy blood & tissue extraction kit (Qiagen; Hilden, Germany). To initially identify hybrids among specimens of the new taxa, DNA concentrations were quantified using a NanoDrop and normalized to approximately 30 ng/μL for amplification of the mitochondrial NADH dehydrogenase subunit 2 (ND2) gene and the internal transcribed spacer 2 (ITS2), following methods described in Freeman *et al.* (2015). Specimens representing the new taxa that had ND2 or ITS2 genes that identified the specimen as a hybrid with a non-native, introduced

bass species were eliminated from further analyses. We then quantified DNA concentrations for 3RAD library preparation using a Qubit 2.0 broad range kit and then normalized concentrations using TBE buffer to a target of 25 ng/μL.

We used the 3RAD Adapterama (Bayona-Vásquez *et al.* 2019) method for genotyping-by-sequencing to identify large numbers of single nucleotide polymorphisms (SNPs) from across the genomes of the two new taxa and six congeners. Library preparation used the enzyme set *MspI*, *BamHI*, and *ClaI*; and multiplexed dual-index custom adapters and iTru primers (Bayona-Vásquez et al. 2019). Sequencing was outsourced to Novogene (novogene.com) to obtain PE150 reads. Returned sequences (NCBI BioProject PRJNA1152248) were demultiplexed and cleaned as in Wares (2024). Paired-end Illumina reads were demultiplexed and cleaned using the *process_radtags* script of Stacks v2 (Catchen *et al.* 2013; Rochette *et al.* 2019) as described in Bayona-Vásquez *et al.* (2019).

To explore the sequence/genotype diversity appropriately before final assembly, we used the Stacks workflow of Toczydlowski *et al.* (2025) to assess a subset of data across multiple assembly parameter combinations of M and n as suggested in Paris *et al.* (2017) and Rochette *et al*. (2019), holding *m* (the number of reads required to call a locus in an individual) at three. We evaluated the parameter M (which characterizes the mismatches allowed between reads within an inferred locus) from 3–7 and the parameter n (which sets the number of mismatches allowed between loci called from other individuals) from M-1, M, and M+1. We evaluated these parameters (Supplement 2, Parameter choice for STACKS) and chose a parameter set of m3-M7-n8 for analysis, as this provided the best assignments across a comparison of eight nominal taxa. After selecting the best-inferred assembly, we exported one random SNP from each locus, and these data were further filtered in R (version 4.3.2; R Core Team 2023) using the package poppr::*missingno* (Kamvar *et al.* 2014) to remove SNPS above a missing cutoff of 0.7 and to remove samples with a missing data cutoff of 0.9, with the goal of removing as much missing data as possible without excluding key populations for the comparison. With this final nuclear SNP set, probability assignments to the eight nominal taxa were made using STRUCTURE version 2.3.4 (Pritchard *et al.* 2000). We used these probability assignments to identify minimally-introgressed specimens of the two new taxa to use for species descriptions and to assess phylogenetic relations. For the phylogenetic analysis, SNPs were exported as a concatenated FASTA sequence alignment and processed in Genious Prime 2021.0.3, with the output from RAxML used as the starting input for Mr. Bayes (4 heated chains of 500,000) to infer statistical support for identified relationships (Huelsenbeck and Ronquist 2001; Ronquist *et al.* 2012).

After identifying non-introgressed individuals and phylogenetic relations based on 3RAD SNP data, we explored population genetic structure within each of the two new taxa to evaluate evidence for non-native status within portions of each taxon's current known range. Specifically, occurrence of Bartram's Bass in the Saluda and Enoree rivers of the Santee River system and of Altamaha Bass in the Ogeechee River (Figure 1) have been hypothesized to have resulted from human introductions in recent decades. To test these hypotheses of non-native status, we applied Discriminant Analysis of Principal Components (DAPC; Jombart *et al.* 2010; Thia 2022) to SNP data for each taxon. The DAPC analysis allowed us to visualize distances in ordination space among individuals collected from differing river or tributary systems including sites where taxon occurrence may represent recent introductions, and to assess the strength of individual assignments to their respective populations. We expected that populations in the Saluda and Enoree systems (for Bartram's Bass) and in the Ogeechee (for Altamaha Bass) would separate from other populations if native, reflecting long-term isolation. We used SNP data for type specimens or for tissue samples with high-probability assignments to their respective taxon (i.e., excluding introgressed individuals) in the DAPC analyses, selected to balance spatial representation across each taxon's current range (Supplement 1). The filtered dataset for Bartram's Bass comprised 36 individuals from seven mainstem or tributary systems, including two (Saluda River, Enoree River) with suspected non-native populations. The filtered dataset for Altamaha Bass comprised 33 individuals collected from the Oconee, Ocumulgee, and (suspected non-native) Ogeechee systems (Supplement 1). We filtered data using R packages SNPfiltR 1.0.1 (DeRaad 2022) and *vcfR* 1.15.0 (Knaus *et al.* 2023) to remove SNPS above a missing cutoff of 0.8 and to remove samples with a missing data cutoff of 0.9. We used the *dapc* command in the R package *adegenet 2.0.0* (Jombart & Collins 2015) to assess genetic discrimination among groups defined *a priori* as the seven populations of Bartram's Bass, and among the three *a priori* populations of Altamaha Bass. For each taxon, we used a-score optimization to select the number of principal components to retain for discriminant analysis, from which we plotted individual scores to visualize genetic differentiation among spatially separated populations.

Specimens utilized for morphological comparisons and type series were drawn from genotyped specimens with a high probability of assignment to Bartram's Bass or Altamaha Bass. A few historical specimens obtained from the Georgia Museum of Natural History D.C. Scott Ichthyology Collection were included that pre-dated the generally believed introduction date of the mid-1980s of non-native *M. henshalli* to Atlantic Slope streams. This arbitrary date excluded from this study many specimens collected during the 1980s and early 1990s. Counts and measurements followed those methods described by Hubbs and Lagler (1958); head length was from the anterior-most point on the upper lip to the posterior bony-end of the operculum, excluding the fleshy membrane. Measurements were made with dial calipers or transferred by dividers to a steel-ruler.

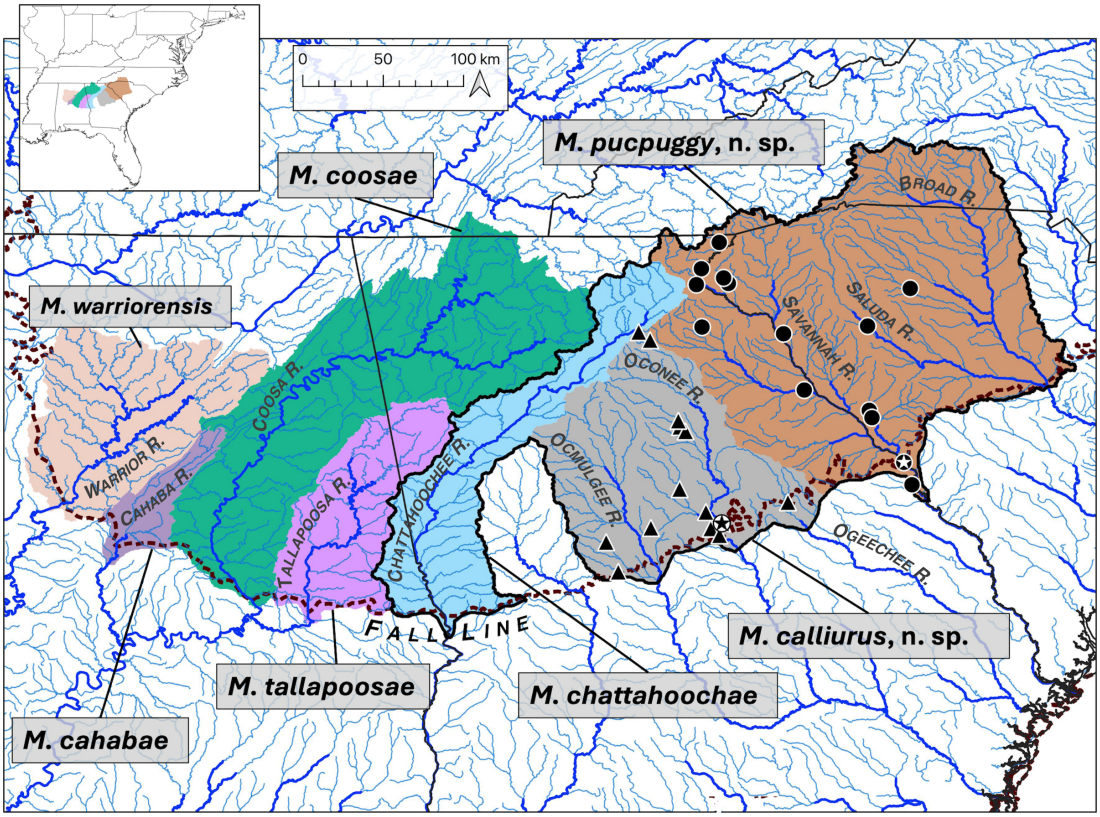

FIGURE 1. The range of the Redeye Bass Clade (comprising, from west to east, *Micropterus warriorensis*, *M. cahabae*, *M. coosae, M. tallapoosae, M. chattahoochae,* and the two taxa described herein, *M. calliurus* **sp. nov.** and *M. pucpuggy* **sp. nov.** The eastern mitochondrial clade identified by Freeman et al. (2015) is outlined in black. The dashed line represents the Fall Line. Locations for type specimens are represented by black triangles for *M. calliurus* and solid black circles for *M. pucpuggy*. The holotype localities for each new taxon are depicted by a circle with a star. The eastern-most locality plotted for *M. pucpuggy* is the Enoree River in the Broad River system.

Results

Genetic Assignments and Phylogeny

Nuclear SNP data provided diagnoses of genetically pure individual specimens based on high assignment probabilities to a single taxon (e.g., STRUCTURE results in Figure 2). We designated 68 contemporary specimens with population assignments based on 3RAD data of 0.96 or higher (58 of 68 had assignments >0.99) to *M. pucpuggy* **sp. nov.** (Bartram's Bass) as types. Contemporary specimens designated as types for *M. calliurus* **sp. nov.** (Altamaha Bass) had assignments based on 3RAD data of 0.95 or higher (61 of 69 type specimens had assignments >0.99). All tissue-only samples used in phylogenetic and population structure analyses (below) had populations assignments >0.99 to the correct taxon. Type material was deposited in the Georgia Museum of Natural History (GMNH) at the University of Georgia and the North Carolina Museum of Natural Sciences (NCSM).

Phylogenetic analysis of 37 individuals representing eight *Micropterus* species (including seven *M. pucpuggy* **sp. nov.** and six *M. calliurus* **sp. nov.**, all types with population assignments of 0.99 or 1.0) illustrated the reciprocal monophyly of the two new taxa and close relative relationships of the new taxa to *M. chattahoochae* and *M. coosae* (Figure 2). The final data set for the phylogeny (Figure 2) was based on 7522 SNPs. All 3RAD data were archived at BioProject PRJNA1152248 (Supplement 3).

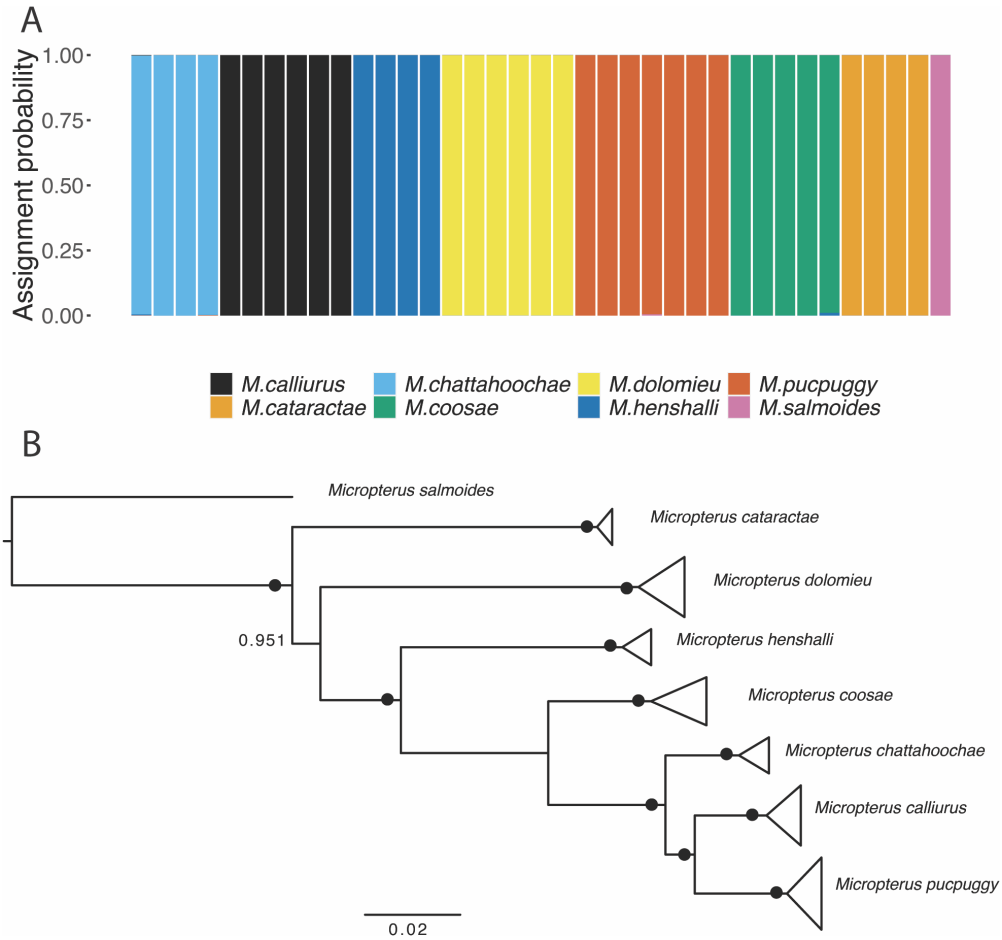

FIGURE 2. (A) Population probability assignments from STRUCTURE (k=8) and (B) inferred maximum credible phylogeny for *Micropterus pucpuggy* **sp. nov.** and *Micropterus calliurus* **sp. nov.** based on a 3RAD data set of 7522 SNPs across 37 individuals representing eight taxa, including one *Micropterus salmoides* as the outgroup. Bootstrap support of 1.0 indicated by black dots.

Population Genetic Structure

The DAPC analysis of population structure among seven mainstem and tributary systems currently occupied by *M. pucpuggy* **sp. nov.** used five principal components from an ordination of 10125 SNP's. The first two axes of the discriminant analyses separated all *a priori* populations (Figure 3A) and assigned all individuals with high confidence to the correct collection locale (Figure 3B). Six individuals from the Saluda River grouped separately from all Savannah River system populations and from two individuals from the Enoree River, suggesting long-term isolation of the Saluda population. The Enoree River fish ordinated close to a Savannah River tributary (Big Generostee Creek) population (Figure 3B), possibly reflecting a more recent introduction from the Savannah system.

The DAPC analysis of population structure among the three major systems occupied by *M. calliurus* **sp. nov.** used one principal component derived from 8837 SNP's. Individuals from the Ocmulgee river system completely separated on this axis from those in the Oconee and Ogeechee systems (Figure 4A) and were unambiguously assigned to the Ocmulgee (Figure 4B). The distribution of individual scores from the Oconee system (which occurs in the same basin as the Ocmulgee, Figure 1) mostly separated from those in the Ogeechee (Figure 4A) although one Ogeechee individual showed majority assignment to the Oconee population (Figure 4B).

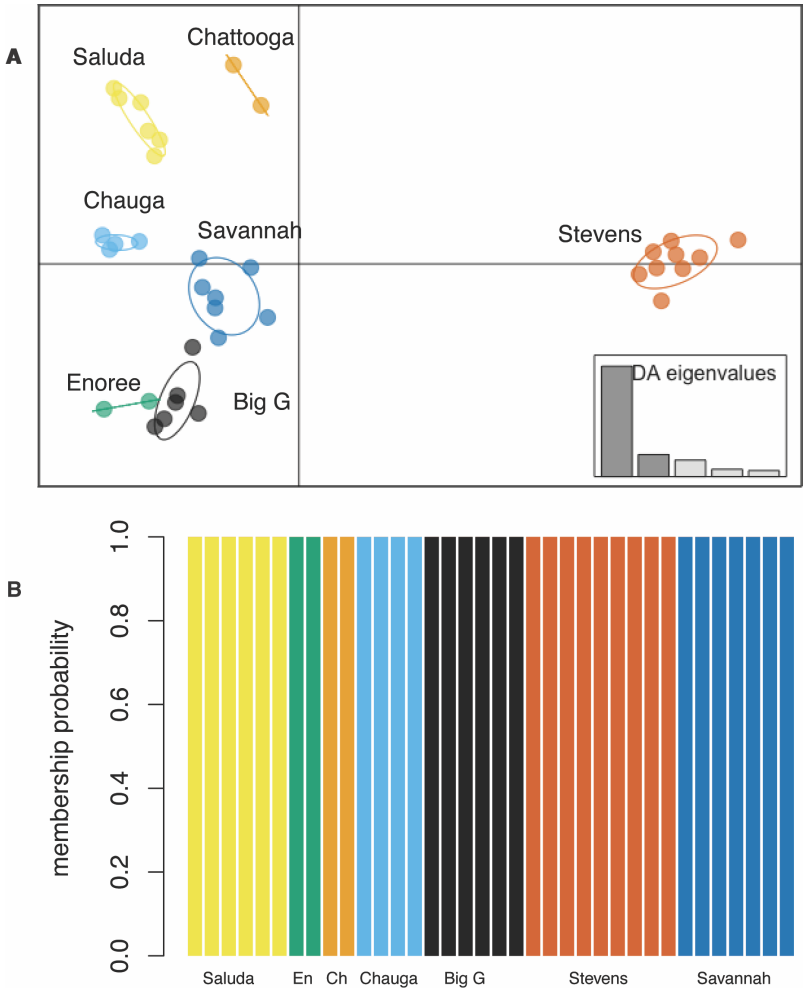

FIGURE 3. (A) Scatterplot of individual *Micropterus pucpuggy* **sp. nov.** representing seven populations projected on the first (x-axis) and second (y-axis) axes derived by DAPC retaining five principal components. (B) DAPC probabilities of population membership for each individual included in the analysis. All 36 assignments were 100% to the correct population. Populations represented are from Big Generostee Creek (Big G), Chattooga River (Ch), Chauga River, Savannah River mainstem ("Savannah"), Stevens Creek, Enoree River (En) and Saluda River.

Taxonomy

Micropterus pucpuggy Freeman & Freeman, new species
Bartram's Bass
Figure 5A, B

Micropterus coosae–Hubbs & Bailey 1940; Lee *et al.* 1980, p. 604; Barwick & Moore 1983; Menhinick 1991, p. 157; Page & Burr 1991, pp. 264–265, map 296; Koppelman & Garrett 2002; Barwick *et al.* 2006; Oswald 2007; Bangs 2011; Bagley *et al.* 2011; Rohde *et al.* 1994; Rohde *et al.* 2009.
Micropterus* sp. cf. *cataractae–Freeman *et al.* 2015; Judson 2018; Taylor *et al.* 2019; Eroh 2020; Tracy *et al.* 2020; Judson *et al.* 2021; Tracy *et al.* 2024.
Micropterus* cf. *coosae–Baker *et al.* 2013; Near & Kim 2021; Kim *et al.* 2022.
Micropterus* sp. cf. *coosae–Bangs *et al.* 2018; Peoples *et al.* 2021; Cox 2022; Gunn *et al.* 2023.

Holotype. **GMNH 6876**, Catalog number 69437, Georgia, Richmond County, Savannah River at Augusta Shoals near Augusta Water Works (33.5145, -82.0007), 194.5 mm SL, 233 mm TL (GMNHTC 16660), 25 April 2023, K. Kubach, M. Scott and A. Gelder, South Carolina Department of Natural Resources (SCDNR) and B.J. Freeman.

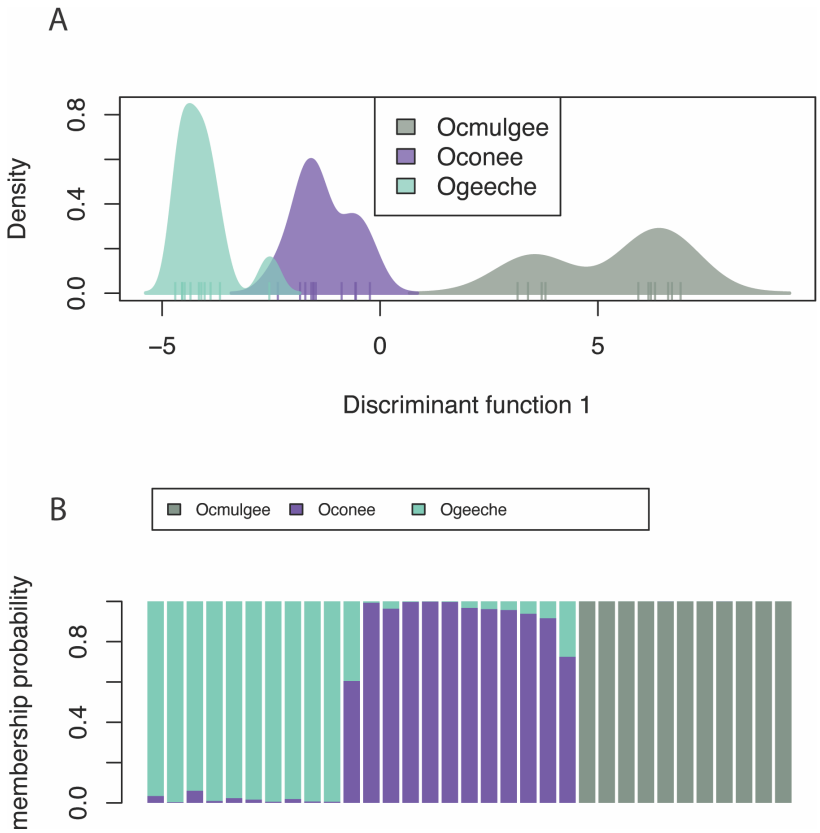

FIGURE 4. (A) Density plot of individual *Micropterus calliurus* **sp. nov.** along the single principal component retained in DAPC analysis of 33 individuals representing three populations. (B) DAPC probabilities of population membership for each individual included in the analysis. Thirty-two of the 33 majority assignments were to the correct population; one Ogeechee River individual had a slightly higher assignment to the Oconee population.

Paratopotypes. GMNH 6877, Catalog number 69439, collected with the holotype, 2 individuals, 217.5–232 mm SL (GMNHTC 16664, 16665), 25 April 2023; **GMNH 6879**, Catalog number 69441, Holotype location from the South Carolina side of the Savannah River (South Carolina, Edgefield County, 33.4947, -81.9908), 7 individuals, 66.0–131.5 mm SL (GMNHTC 16667, 16670, 16672, 16673, 16675, 16677, 16679), 25 May 2023, B.J. Freeman, K. Kubach, D. Gelder, R. Moore, K. Lusk, SCDNR. **NCSM 117008**, same collection as GMNH 6879, 2 individuals, 74–145 mm SL (GMNHTC 16668, 16676).

Paratypes. GMNH 6881, Catalog Number 69443, South Carolina, Oconee County, Chauga River near Chau Ram County Park (34.6820, -83.1465), 10 individuals, 45–231.5 mm SL (GMNHTC 16691, 16694, 16695, 16699, 16714, 16735, 16737,16744, 16745, 16750), 25 August 2022, SCDNR; **GMNH 6882**, Catalog Number 69444, South Carolina, Oconee County, Chauga River at Chau Ram Park (34.6818, -83.1464), 6 individuals, 81.7–127.4 mm SL (GMNHTC 12201,12202, 12203, 12205,12208, 12211), 1995, SCDNR; **GMNH 6883**, Catalog Number 69445, South Carolina, Oconee County, Chauga River at Cobb Bridge (34.7179, -83. 1772), 7 individuals, 41.5–145 mm SL (GMNHTC 16704, 16708, 16717, 16721, 16731, 16732, 16734), 31 August 2022, SCDNR; **GMNH 6884**, Catalog Number 69446, South Carolina, Anderson County, Big Generostee Creek at Broadwell Mill Road (34.3535, -82.7860), 6 individuals, 60–160 mm SL (GMNHTC 16522, 16523, 16524,16529, 16531, 16541), 3 August 2022, SCDNR; **GMNH 6885**, Catalog Number 69447, South Carolina, Greenwood and Laurens Counties, Saluda River at Ware Shoals alongside Power House Road (34.4039, -82.2373), 5 individuals, 67–149 mm SL (GMNHTC 16547, 16548, 16569, 16574, 16577), 9 August 2022, B.J. Freeman, K. Kubach, D. Gelder, M. Limehouse, P. Carson, I. Tiller, M. Scott, SCDNR; **NCSM 117009**, Same collection as GMNH 6885, 2 individuals, 72–144 mm SL (GMNHTC 16550, 16564); **GMNH 6896**, Catalog number 69458, South Carolina, Laurens County, Enoree River downstream of Riverdale Dam (34.6493, -81.9591), 1 individual, 70 mm SL (GMNHTC 16649), 15 August 2022, B.J. Freeman, K. Kubach, D. Gelder, I. Tiller, M. Scott, SCDNR; **GMNH 6886**, Catalog Number 69448, South

Carolina, McCormick County, Stevens Creek at State Road 283 E Plum Branch (33.8535, -82.2268), 10 individuals, 99.9–191.6 mm SL (GMNHTC 12224–12226, 12228, 12230, 12232, 12235–12238), 23 September 1996, SCDNR; **GMNH 5615**, Catalog number 53850, South Carolina, McCormick County, Stevens Creek at State Road S-33-21 (Upper Mill Road), 6.7 km SE of Plum Branch SC and 2.4 km NNE of Parksville SC, city centers (33.8058, -82.2089), 5 individuals, 50–174 mm SL (GMNHTC 12137–12141), 20 September 2013, B.J. Freeman, J. Leitner, C. Poeta; **GMNH 5138**, Catalog number 50732, Georgia, Elbert and Wilkes Counties, Broad River at Anthony Shoals, 15.6 air km NE of Tignall, GA city center (33.9862, -82.6508), 1 individual, 116 mm SL (GMNHTC 10569), 4 March 2011, B.J. Freeman, C.A. Straight, M.M. Hagler; **GMNH 6895**, Catalog number 69457, Georgia, Rabun County, Chattooga River near Daniel Creek, 8.6 km NE of Tallulah Falls GA city center (34.7781, -83.3226), 3 individuals, 152–194 mm SL (GMNHTC 12146–12148), 29 September 2013, B.J. Freeman, M.C. Freeman, J. Nelson.

Paratypes, Historical Specimens. GMNH 500, Catalog number 3964, Georgia, Rabun County, West Fork Chattooga River at first bridge above Warwoman Road (34.9495, -83.2060), 1 individual, 150 mm SL, 25 July 1957, T.J. Merkel, V.C. Williams; **GMNH 1249**, Catalog number 7695, Georgia, Stephens County, Panther Creek in Savannah Drainage (34.6740, -83.3547), 1 individual, 193 mm SL, 19 June 1969, M. Seehorn; **GMNH 700**, Catalog number 5360, Georgia, Elbert and Wilkes Counties, Broad River at Anthony Shoals (33.9862, -82.6508), 1 individual, 234 mm SL, 10 November 1959, Frey, Bryan, Aderhold; **GMNH 696**, Catalog number 5289, Georgia, Franklin County, Middle Fork Broad River, Station #1 NW of Carnesville (34.3972, -83.3185), 1 individual 60.5 mm SL, 23 September 1959, Frey, Aderhold, Smisson; **GMNH 694A**, Catalog number 5277, Georgia, Richmond County, Savannah River, Station #2 below lock and dam near Bush Field (33.3684, -81.9474), 2 individuals 140.5–159.0 mm SL, 10 September 1959, Frey, Aderhold, Bryan.

Diagnosis. *Micropterus pucpuggy* is a monophyletic, divergent genetic lineage of bass distinguished from other species of *Micropterus* by the following combination of characters: shallow separation between the spinous- and soft-dorsal fins, glossohyal tooth patch usually present, sides patterned with elongated lateral blotches that do not form a lateral band and that intersect ventrally with rows of dark spots, and with a suffusion of rosy-pink color in the soft-dorsal, anal and caudal fins. In contrast, *M. salmoides* and *M. nigricans* have a deep notch between the spinous- and soft-dorsal fins, with the shortest posterior spine <50% length of longest (Etnier & Starnes 1993). *Micropterus punctulatus* and *M. henshalli* have short lateral blotches that form an irregular, lateral band (Hubbs & Bailey 1940). *Micropterus dolomieu* and *M. velox* may have discernable lateral bars but lack ventral rows of spots along the sides (Hubbs & Bailey 1940, Etnier & Starnes 1993). *Micropterus cataractae* and *M. warriorensis* typically lack a tooth patch (Williams & Burgess 1999; Baker *et al.* 2013). *Micropterus treculii, M. notius, M. cahabae* and *M. tallapoosae* lack chromatic color in dorsal or anal fins (Bailey & Hubbs 1949; Baker *et al.* 2013, Robins *et al.* 2018). *Micropterus coosae* has brick-red color in the soft dorsal, anal and caudal fins; juveniles have elongated lateral blotches but these become faint with age, especially posteriorly (Hubbs & Bailey 1940; Baker *et al.* 2013).

Micropterus pucpuggy differs from *M. chattahoochae* in having a modally higher count of lateral-line scale rows (70–71 versus 63–66, Baker *et al.* 2013), lateral blotches that extend to 5–9 scale rows below the mid-line (compared to fewer than five scale rows below the mid-line), and in lacking intense bright orange color on the caudal and anal fins (compared with bright orange on the upper and lower caudal rays and that may wrap around the distal edge of the caudal fin forming a marginal band, and bright orange in the anal fin that extends from the anterior spines and suffuses the distal 2/3rd of the first 7–8 inter-radial membranes). The caudal fin margin of *M. pucuggy* is never chromatically colored with orange as in *M. chattahoochae*.

Micropterus pucpuggy differs from *M. calliurus* **sp. nov.** in caudal- and anal-fin coloration. *M. calliurus* has salmon-orange color in the tips of first three upper and lower caudal rays and membranes (absent in *M. pucpuggy*) and in the distal one-half of the first five inter-radial membranes of the anal fin (in contrast to a diffuse rosy-pink or no chromatic color in the distal anterior anal-fin membranes of *M. pucpuggy*). Meristically, *M. pucpuggy* has modally higher counts of lateral line scale rows (70–71 vs. 69–70; Table 1), lateral scale rows above (9–10 vs. 8–9; Table 2) and below (16–18 vs. 15–16: Table 3) the lateral line, and cheek scale rows (14–15 vs. 12–13; Table 4) than *M. calliurus*. *Micropterus pucpuggy* has a greater relative body depth (253–323 thousandths of SL, mean 289 vs. 251–300, mean 276) and caudal peduncle depth (113–136, mean 123 vs. 109–130, mean 116) than *M. calliurus* (Figure 6; Table 5). In addition to being deeper bodied, *M. pucpuggy* has a longer relative caudal peduncle (207–246, mean 227 vs. 205–241, mean 221, $p<0.002$; weakly correlated with SL, $r < 0.2$ in both taxa), and shorter relative predorsal length (386–446, mean 412 vs. 395–432, mean 418, $p<0.03$; similarly correlated with SL, $r = -0.25$ and -0.32), compared with *M. calliurus* in the type series.

TABLE 1. Pored lateral line scale counts for *Micropterus pucpuggy* **sp. nov.** from the Savannah and Santee River drainages, and *Micropterus calliurus* **sp. nov.** from the Altamaha and Ogeechee River drainages. Counts for each species are followed by counts for individual river systems.

	Pored Lateral Line Scales																
	63	64	65	66	67	68	69	70	71	72	73	74	75	76	N	Mean	SD
Micropterus pucpuggy	1			2	8	4	9	14	14	8	4	1	1	3	69	70.18	2.44
Santee					1	1	1	1	1		1			2	8	71.25	3.45
Savannah	1			2	7	3	8	13	13	8	3	1	1	1	61	70.04	2.27
Micropterus calliurus		1	7	2	8	7	12	10	5	5	1	1			59	68.69	2.29
Altamaha		1	4	1	5	6	9	8	4	5	1	1			45	69.00	2.31
Ogeechee			3	1	3	1	3	2	1						14	67.71	2.02

TABLE 2. Scale row counts above the lateral line for *Micropterus pucpuggy* **sp. nov.** from the Savannah and Santee River drainages, and *Micropterus calliurus* **sp. nov.** from the Altamaha and Ogeechee River drainages. Counts for each species are followed by counts for individual river systems.

	Scale Rows Above Lateral Line							
	7	8	9	10	11	N	Mean	SD
Micropterus pucpuggy		8	32	27	2	69	9.33	0.72
Santee			3	5		8	9.62	0.52
Savannah		8	29	22	2	61	9.30	0.74
Micropterus calliurus	1	25	29	2		57	8.56	0.60
Altamaha	1	23	18	1		43	8.44	0.59
Ogeechee		2	11	1		14	8.93	0.47

TABLE 3. Scale row counts below the lateral line for *Micropterus pucpuggy* **sp. nov.** from the Savannah and Santee River drainages, and *Micropterus calliurus* **sp. nov.** from the Altamaha and Ogeechee River drainages. Counts for each species are followed by counts for individual river systems.

	Scale Rows Below Lateral Line								
	14	15	16	17	18	19	N	Mean	SD
Micropterus pucpuggy		12	25	10	17	5	69	16.68	1.23
Santee			2	2	3	1	8	17.38	1.06
Savannah		12	23	8	14	4	61	16.59	1.23
Micropterus calliurus	7	20	26	5			58	15.50	0.82
Altamaha	7	17	16	4			44	15.39	0.87
Ogeechee		3	10	1			14	15.86	0.53

TABLE 4. Cheek scale row counts for *Micropterus pucpuggy* **sp. nov.** from the Savannah and Santee River drainages, and *Micropterus calliurus* **sp. nov.** from the Altamaha and Ogeechee River drainages. Counts for each species are followed by counts for individual river systems.

	Cheek Scale Rows										
	10	11	12	13	14	15	16	17	N	Mean	SD
Micropterus pucpuggy		1	3	11	19	15	3	1	53	14.08	1.14
Santee				1	3	2	1		7	14.43	0.98
Savannah		1	3	10	16	13	2	1	46	14.02	1.16
Micropterus calliurus	1	7	15	19	10	3			55	12.71	1.13
Altamaha	1	7	13	16	3	1			41	12.39	1.02
Ogeechee			2	3	7	2			14	13.64	0.93

TABLE 5. Proportional measurements for type specimens of *M. calliurus* **sp. nov.** (all from the Altamaha River system) and *M. pucpuggy* **sp. nov.** (seven from the Saluda River and up to 32 from the Savannah River system), expressed as thousandths of standard length except as noted.

		M. calliurus		*M. pucpuggy*
Measurement	n	Mean (range)	n	Mean (range)
Standard length	37	151 (89-220)	39	137 (58-234)
Head length	37	328 (302-349)	39	329 (304-366)
Greatest body depth	36	276 (251-300)	39	289 (253-323)
Least caudal peduncle depth	36	116 (109-130)	39	123 (113-136)
Caudal peduncle length	37	221 (205-241)	39	227 (207-246)
Highest dorsal spine	36	76 (61-91)	38	81 (59-107)
Predorsal length	36	418 (395-432)	39	412 (386-446)
Snout length (to Head length)	37	297 (253-326)	39	296 (230-331)
Penultimate dorsal spine (to highest spine)	36	723 (600-857)	38	693 (571-885)

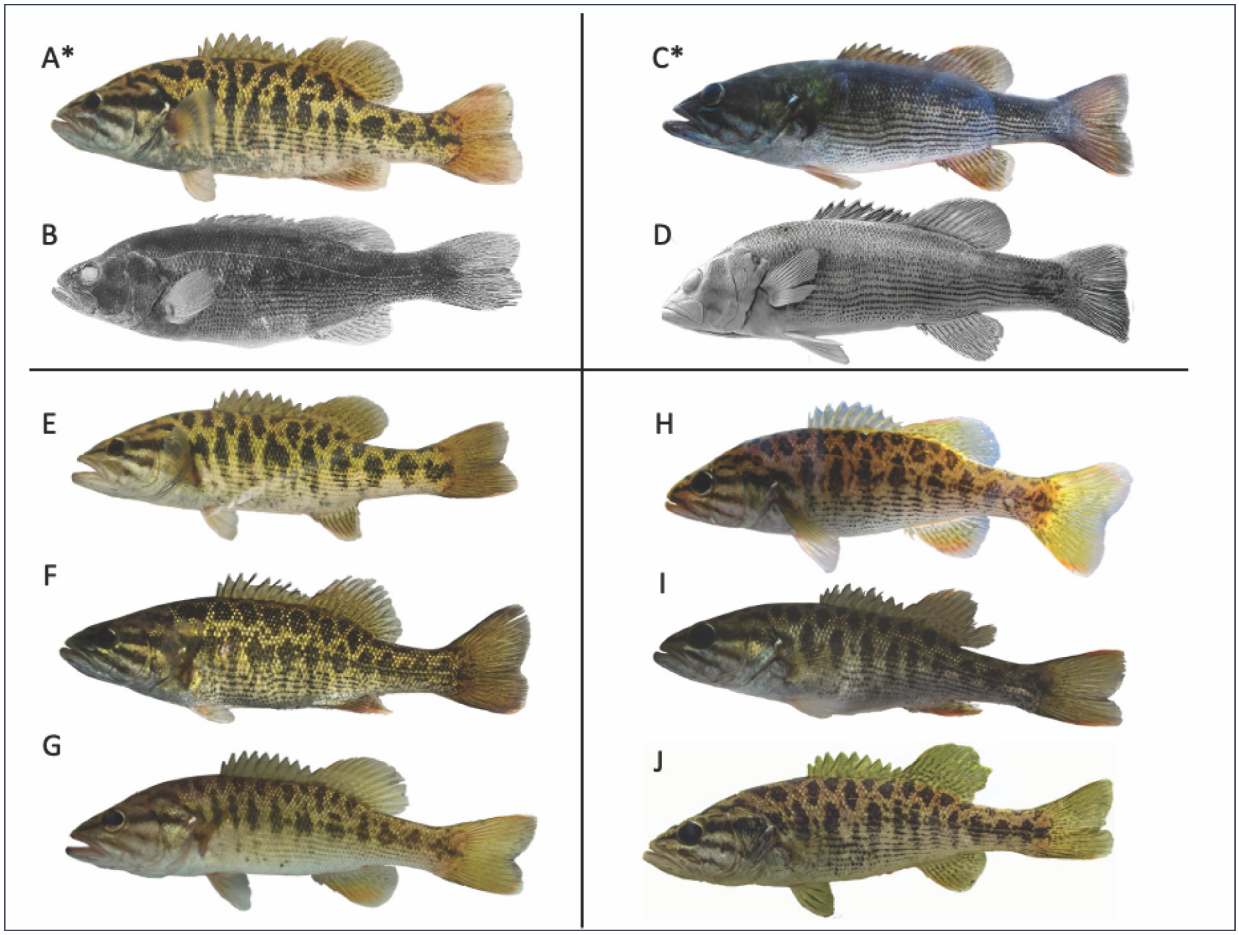

FIGURE 5. (A, B) Holotype of *Micropterus pucpuggy* **sp. nov.** (GMNH Catalog no. 69437, 194.5 mm SL; A, in life; B, in alcohol). (C, D) Holotype of *Micropterus calliurus* **sp. nov.** (GMNH Catalog no., 54394, 176.6 mm SL; C, in life; D, in alcohol). (E) *Micropterus pucpuggy* paratopotype (GMNH Catalog no. 69439, 217.5 mm SL). (F, G) *M. pucpuggy* x *M. henshalli* hybrids from (F) same locale as holotype (GMNHTC 16663, 222 mm SL) and (G) from Chauga River (GMNHTC 16748, 161.5 mm SL). (H, I, J) *Micropterus calliurus* paratypes from Little River (H; GMNH Catalog no. 53907, 129.8 mm SL), Jack's Creek (I; NCSM 117010, 120.25 mm SL), and Tobesofkee Creek (J; GMNH Catalog no. 69454, 182.25 mm SL). Asterisks denote holotypes.

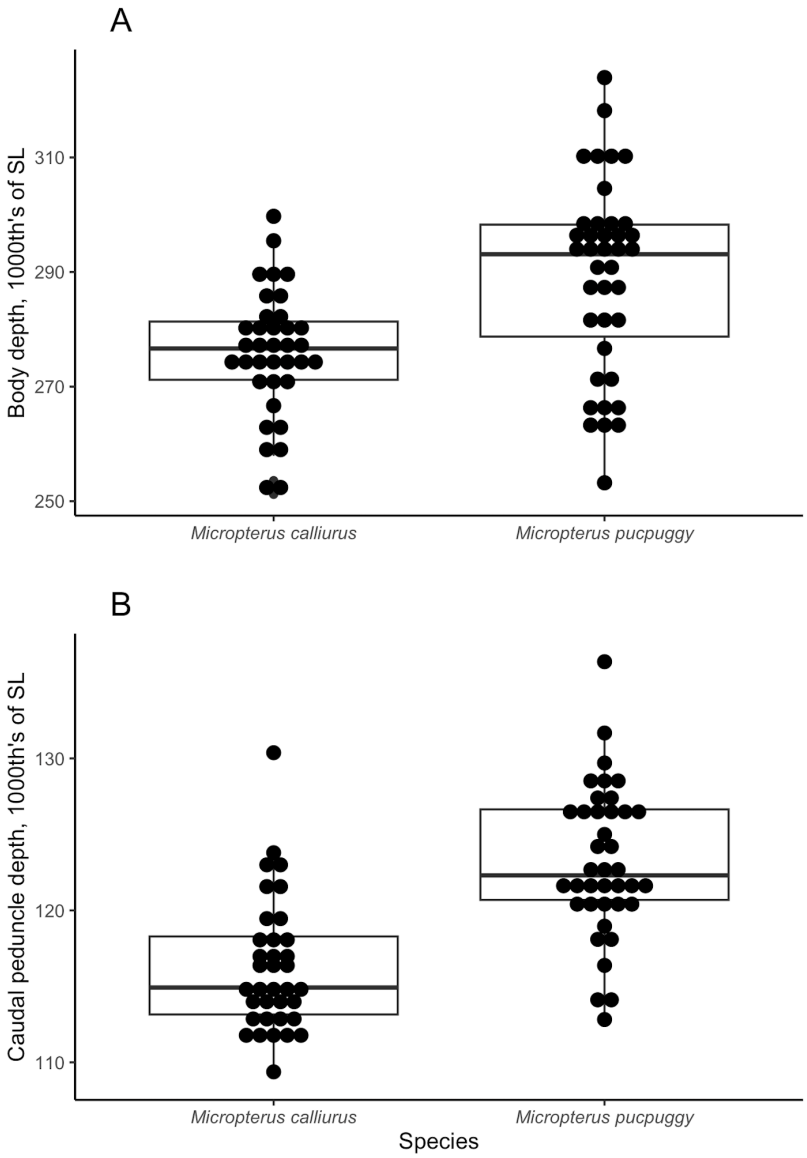

FIGURE 6. (A) Greatest body depth and (B) least caudal peduncle depth for *Micropterus calliurus* **sp. nov.** (n=36) and *Micropterus pucpuggy* **sp. nov.** (n=39) expressed as one-thousandths of standard length (SL). Means for each of these measurements differ significantly between *M. calliurus* and *M. pucpuggy* (two-sample t-tests; $p<0.001$). Boxes and lines show median, interquartile and 1.5 x interquartile values, with individual data plotted as points. Neither body depth nor caudal peduncle depth correlate strongly with standard length (r values = 0.30 and 0.02 for body depth:SL vs SL, and 0.11 and -0.17 for caudal peduncle depth: SL vs SL, for *M. calliurus* and *M. pucpuggy*, respectively).

Description. Relatively robust bass; greatest body depth of holotype 57.5 mm (296 thousandths of SL). Standard length to at least 274 mm (GMNH 4790, Chattooga River, 1969); largest type specimen 234 mm SL (GMNH 700, Broad River at Anthony Shoals, Elbert-Wilkes Co., GA). Scale and fin element counts provided in Tables 1–4 and 6–10, and proportional measurements in Table 5. Modes for counts as follows: lateral line scale rows 70–71 (most, 67–73); dorsal spines 10; dorsal rays 12; pectoral rays 15–16; anal spines 3; anal rays 10; scales above the lateral line 9–10; scales below the lateral line 16–18; caudal peduncle scale rows 30 (14–15 above the lateral line, 13–14 below the lateral line), cheek scale rows 13–15. Shallow notch between the spinous- and soft-dorsal fins present with length of shortest posterior spine averaging 69% (n=38) of length of longest spine. Oval tooth patch usually present (47 of 53 type specimens examined).

Color in life. Sides of juveniles and adults strikingly patterned with 11 lateral blotches with greatest widths of four to six scales. First lateral blotch confluent with, and extension of, blotch extending from temporal region

to below pectoral girdle. Lateral blotches do not form lateral stripe and separated by two to three scales between each blotch. Lateral blotches typically extend one to three scale rows dorsally across the lateral-line scales. Lateral blotches extend below midline, where they diffusely intersect ventral-lateral rows of spots, which number modally seven to 10. Series of irregularly shaped dorsolateral botches extend from base of dorsal fins down to or between lateral blotches; dorsolateral blotches may or may not touch dorsal-fin base.

Scales above midline of the body, not in lateral blotch, light gold in color. Scales below midline with dark, narrow, medial coloration forming series of parallel rows of spots. Scales in the lateral blotches outlined in black with gray to black centers. Distal portion of opercular flap with bright silver coloration extending one scale above and below tip of opercle. Pectoral-fin rays ventrally outlined with black melanophores forming thin black margin. Pelvic fins mottled with discrete melanophores basally; the distal portion of fin immaculate and clear. Head marked by three bold black stripes. First stripe three to five scale rows in vertical extent and tapers as it extends from tip of opercle through eye, narrowing on snout and stopping after nares. Middle stripe on head extends from above angle of preopercle and angles dorsally to orbit, stripe four or five scale rows in height. Lower head stripe extends anteriorly from below angle of preopercle and crosses dorsal portion of maxilla; this stripe two or three scale rows in height and diffuse posteriorly, intensifying as it crosses preopercle onto maxilla. Medial posterior and anterior regions of iris deep carmine-red; black pupil ringed with thin gold margin on iris. Scales on opercle between three stripes grayish-blue and this coloration extends onto mandible and gular region. Belly and breast appear mottled as some scales completely dark while others white in patches. Anal fin variously patterned with inter-radial membranes grading from tightly spaced melanophores to discreetly spotted on last six membranes, although distal one-third of posterior portion of fin typically lacks pigment. First four or five rays may be suffused with rosy-pink in distal anterior membranes or have no chromatic coloration. Anal spines heavily pigmented with melanophores. Caudal-fin rays and inter-radial membranes variously pigmented with suffusion of rosy-pink except for distal margin. Upper and lower caudal rays outlined in black, with first three membranes being immaculate and appearing white. Caudal base variously stitched in row of melanophores. Basicaudal spot three or four scale rows in height, not discrete, and extends anteriorly nine to 10 scales. Spinous-dorsal fin inter-radial membranes suffused with dark melanophores. Soft-dorsal fin inter-radial membranes have discrete concentrations of dark melanophores providing a banded appearance that grades to more uniform dark distally. Posterior distal portion of soft dorsal may be suffused with rosy-pink.

Color in alcohol. Holotype preserved in 70% ethanol much darker than in life, with lateral and dorsolateral blotches difficult to discern. Lateral and dorsolateral markings often obscure in preserved specimens. Pores in the lateral-line scales light in color, in contrast to the darker scale background.

Distribution. *Micropterus pucpuggy* currently occurs in the upper Savannah Basin in Georgia and South Carolina, east to the Broad River system of the Santee Basin in the Carolinas (Figure 1). *Micropterus pucpuggy* occurs primarily above the Fall Line (the physiographic boundary between Piedmont uplands and Coastal Plain); however, in the Savannah River *Micropterus pucpuggy* is routinely collected downstream of the Fall Line in suitable shoal habitat (Rohde *et al.* 2009). Genetically pure *Micropterus pucpuggy* are extant in all major watersheds in their currently known range, however introgressive hybridization with *Micropterus henshalli* has caused declines and localized extirpation in smaller constituent systems (Leitner *et al.* 2015; Bangs *et al.* 2018; Peoples *et al.* 2021).

Whereas the native range of *M. pucpuggy* unambiguously includes the mainstem and tributaries of the Savannah River system, native status in the upper Santee Basin (the Saluda and Broad River systems) is less certain. Documented occurrence of *M. pucpuggy* in the Santee Basin prior to 1980 appears limited to perhaps two records in the Saluda River (shown as *M. coosae* in Lee *et al.* 1980), suggesting that wider present-day occurrence in the Saluda River (Rohde *et al.* 2009) and in the Broad River system (including the Enoree River in South Carolina) resulted from introductions. Tracy *et al.* (2020) attributed occurrence in the headwaters of the Green River (Broad River system) in North Carolina to an introduction. Close positioning of two Enoree River specimens with Savannah River specimens in our DAPC analysis (Figure 3) also suggests that Broad River system populations of *M. pucpuggy* may have resulted from introductions. In contrast, we interpret the clustering and separation of Saluda River specimens from all Savannah River populations in the DAPC analysis (Figure 3) as strong evidence for long isolation of the Saluda populations from those in the Savannah, and likely native status. An historic record of *M. pucpuggy* (identified as *Micropterus coosae*) from Georges Creek (26 July 1954, USNM 237787), a tributary to what is now Lake Saluda (north of Greenville South Carolina) further suggests that at least the Saluda River system in the upper Santee Basin is within the native range of *M. pucpuggy*. The headwaters of the Savannah and Saluda

systems share at least 16 other upland fish species—as illustrated in Rhode *et al.* (2009) and in GMNH records for *Cyprinella pyrrhomelas* (Cope)—and we think it is reasonable that Bartram's Bass also occurs natively in both systems.

Etymology. The trivial epithet *pucpuggy* honors the Seminole-Creek inhabitants of Florida, whose Chief bestowed the name "Puc Puggy", meaning the "Flower hunter", on William Bartram (referenced by Bartram in Part Two pp. 117–118. [pp. 184, 185 in First Edition of the Travels], as the Chief of Cuscowilla, called the Cowkeeper, "… saluting me by the name of Puc Puggy or the Flower hunter…"; Harper 1958). William Bartram's travels in 1773-1776 brought him through the native range of *M. pucpuggy*, where he described flora and fauna including fishes previously unknown to North American colonists (Berra 1989). Treated as a noun in apposition.

Micropterus calliurus Freeman and Freeman, new species
Altamaha Bass
Figure 5C, D

Micropterus coosae–Lee *et al.* 1980, p. 604; Page & Burr 1991, pp. 264–265, map 296; Koppelman & Garrett 2002; Oswald *et al.* 2015.
Micropterus* cf. *coosae–Baker *et al.* 2013; Near & Kim 2021; Kim *et al.* 2022.
Micropterus* sp. cf. *coosae–Judson 2018.
Micropterus* sp. cf. *cataractae–Freeman *et al.* 2015; Taylor *et al.* 2019; Eroh 2020.

Holotype. GMNH 5752, Catalog number 54394, Georgia, Baldwin County, Champion Creek downstream of Georgia College Field Station (33.1163, -83.1890), 176.6 mm SL, 214 mm TL (GMNHTC 12240), 24 June 2014, Chris Skelton.

Paratopotype. **GMNH 5751**, Catalog number 54393, 73.6 mm SL (GMNHTC 12219),16 February 2012, D.L. Parmley.

Paratypes. GMNH 6888, Catalog number 69450, Georgia, Hall County, North Oconee River at Whitehall Road (37.3644, -83.7316), 4 individuals, 107–171.5 mm SL (GMNHTC 12156–12159), 16 October 2013, S.R. Dodd and Nutter and Associates; **GMNH 6889**, Catalog number 69451, Georgia, Warren County, Ogeechee River above Shoals Road, 5.1 air km SSW Jewel (33.2542, -82.7573), 23 individuals, 52.3–173.5 mm SL (GMNHTC 12163–12173, 12175–12178, 12180–12185, 12187, 12190), 15 September 2006, J. Quattro, students, and Georgia Department of Natural Resources (GDNR); **GMNH 6901**, Catalog number 69463, Georgia, Warren County, Ogeechee River above Shoals Road, 5.1 air km SSW Jewel (33.2542, -82.7573), 1 individual, 204 mm SL (GMNHTC 14291), 12 September 2020, J.R. Bennett and E. Dwoskin; **GMNH 6890**, Catalog number 69452, Georgia, Morgan County, Jack's Creek downstream Wagnon Mill Road crossing (33.7457, -83.4627), 11 individuals, 54–205.5 mm SL (GMNHTC 17314–17320, 17322–17325), 27 October 2023, B.J. Freeman, M.C. Freeman, B. Albanese, B.G. Hilburn, C. Kaiser, GDNR; **NCSM 117010**, Same collection as GMNH 6890, 2 individuals, 122–183 mm SL (GMNHTC 17313, 17321); **GMNH 6902**, Catalog number 69464, Georgia, Morgan County, Jack's Creek downstream Wagnon Mill Road crossing (33.7457, -83.4627), 1 individual, 117 mm SL (GMNHTC 9213), 29 June 2010, B.J. Freeman; **GMNH 6891**, Catalog number 69453, Georgia, Jones County, Caney Creek upstream of Pippin Road (33.0867, -83.6523), 6 individuals, 55–155 mm SL (GMNHTC 17328, 17331, 17333-17336), 14 December 2023, B.J. Freeman, M.C. Freeman, B. Albanese, B.G. Hilburn, C. Kaiser, GDNR; **NCSM 117011**, Same collection as GMNH 6891, 2 individuals, 102–152 mm SL (GMNHTC 17329, 17330); GMNH **6892**, Catalog number 69454, Georgia, Monroe County, Tobesofkee Creek (32.9936, -83.9439), 4 individuals, 131.25–220 mm SL, (GMNHTC 17337–17340), 15–16 May 2024, B. Bowen and E. Dodsan; **GMNH 6893**, Catalog number 69455, Georgia, Morgan and Oconee Counties, Apalachee River at Potleaf Shoals (33.7162, -83.4258), 5 individuals, 152.5–209 mm SL (GMNHTC 17341–17344, 17346), 29–31 May 2024, B. Bowen; **GMNH 6897**, Catalog number 69459, Georgia, Morgan and Oconee Counties, Apalachee River upstream of Price Mill Road (33.7858, -83.4729), 2 individuals 192.5–183.3 mm SL (GMNHTC 10349,10350), 22 July 2010, B.J. Freeman and D.M. Walters; **GMNH 5624**, Catalog number 53907, Georgia, Putnam County, Little River at Martin Mill Road, 7.2 km WNW of Eatonton GA city center (33.3395, -83.4646), 2 individuals, 129.8–186 mm SL (GMNHTC 12152,12153), 12 October 2013, M.C. Freeman, B.J. Freeman, E. Horton, A. Pope; **GMNH 6899**, Catalog number 69461, Georgia, Baldwin County, Fishing Creek at US Hwy 441 bypass (33.0824, -83.2612), 1 individual, 106.8 mm SL (GMNHTC 12216), 17

October 2013, C.E. Skelton; **GMNH 6900**, Catalog number 69462, Georgia, Baldwin County, Fishing Creek at US Hwy 441 bypass (33.0824, -83.2612), 3 individuals, 53.7–145.4 mm SL (GMNHTC 12220,12221, 12222), 22 November 2013, C.E. Skelton.

Paratypes, Historical Specimens. GMNH 433, Catalog number 3493. Georgia, Putnam County, Sinclair Reservoir, GA Hwy 24 at Little River (33.1892, -83.2921), 1 individual, 144 mm SL, May 1955, Wright through E.C. Kinney; **GMNH 664**, Catalog number 5046. Georgia, Crawford County, Echeconnee Creek (32.7997, -83.8650), 2 individuals, 133–164 mm SL, 7 June 1959, Hastings; **GMNH 1456**, Catalog number 9407. Georgia, Hall County, Candler Creek at GA Hwy 52 (34.3115, -83.6569), 1 individual, 128 mm SL, 16 April 1980, B.J. Freeman, J. Rappole, C. Teplis; **GMNH 1456A,** Catalog number 9399. Georgia, Hall County, Candler Creek at GA Hwy 52 (34.3115, -83.6569), 1 individual, 50 mm SL, 8 May 1981, D.C. Scott, B.J. Freeman *et al.*; **GMNH 2398**, Catalog number 14286, Georgia, Baldwin County, Oconee River upstream from GA Hwy 22/24 bridge (33.0852, -83.2143), 1 individual, 248 mm SL, 13 March 1992, B.J. Freeman, J. Evans.

TABLE 6. Dorsal-fin spine and ray counts for *Micropterus pucpuggy* **sp. nov.** from the Savannah and Santee River drainages, and *Micropterus calliurus* **sp. nov.** from the Altamaha and Ogeechee River drainages. Counts for each species are followed by counts for individual river systems.

	Dorsal-Fin Spines						Dorsal-Fin Rays						
	9	10	11	N	Mean	SD	8	11	12	13	N	Mean	SD
Micropterus pucpuggy	3	64	2	69	9.98	0.27	1	4	56	8	69	12.00	0.64
Santee		8		8	10.00	-			8		8	12.00	-
Savannah	3	56	2	61	9.98	0.29	1	4	48	8	61	12.00	0.68
Micropterus calliurus	3	54	1	58	9.96	0.26			55	4	59	12.07	0.25
Altamaha	1	42	1	44	10.00	0.22			42	3	45	12.07	0.25
Ogeechee	2	12		14	9.86	0.36			13	1	14	12.07	0.27

TABLE 7. Pectoral-fin ray counts for *Micropterus pucpuggy* **sp. nov.** from the Savannah and Santee River drainages, and *Micropterus calliurus* **sp. nov.** from the Altamaha and Ogeechee River drainages. Counts for each species are followed by counts for individual river systems.

	Pectoral-Fin Rays					
	15	16	17	N	Mean	SD
Micropterus pucpuggy	22	39		61	15.64	0.48
Santee	4	4		8	15.50	0.53
Savannah	18	35		53	15.66	0.48
Micropterus calliurus	17	35	2	54	15.72	0.53
Altamaha	12	28	2	42	15.76	0.53
Ogeechee	5	7		12	15.58	0.51

TABLE 8. Anal-fin spine and ray counts for *Micropterus pucpuggy* **sp. nov.** from the Savannah and Santee River drainages, and *Micropterus calliurus* **sp. nov.** from the Altamaha and Ogeechee River drainages. Counts for each species are for all type specimens followed by counts for individual river systems.

	Anal-Fin Spines					Anal-Fin Rays					
	2	3	N	Mean	SD	9	10	11	N	Mean	SD
Micropterus pucpuggy	1	68	69	2.98	0.12	5	54	10	69	10.07	0.46
Santee		8	8	3.00	-		6	2	8	10.25	0.46
Savannah	1	60	61	2.98	0.13	5	48	8	61	10.04	0.46
Micropterus calliurus		57	57	3.00	-	9	45	3	57	9.89	0.45
Altamaha		43	43	3.00	-	7	33	3	43	9.91	0.48
Ogeechee		14	14	3.00	-	2	12		14	9.86	0.36

TABLE 9. Caudal-peduncle scale row counts above and below the lateral line for *Micropterus pucpuggy* **sp. nov.** from the Savannah and Santee River drainages, and *Micropterus calliurus* **sp. nov.** from the Altamaha and Ogeechee River drainages. Counts for each species are followed by counts for individual river systems

	Caudal Peduncle Scale Rows Above Lateral Line						Caudal Peduncle Scale Rows Below Lateral Line					
	13	14	15	N	Mean	SD	13	14	15	N	Mean	SD
Micropterus pucpuggy	11	36	22	69	14.16	0.68	16	43	10	69	13.91	0.61
Santee		8		8	14.00	-	2	6		8	13.75	0.46
Savannah	11	28	22	61	14.18	0.72	14	37	10	61	13.93	0.63
Micropterus calliurus	27	27	4	58	13.60	0.62	25	32	1	58	13.59	0.53
Altamaha	25	15	4	44	13.52	0.66	22	21	1	44	13.52	0.55
Ogeechee	2	12		14	13.86	0.36	3	11		14	13.79	0.43

TABLE 10. Total scale row counts around the caudal peduncle for *Micropterus pucpuggy* **sp. nov.** from the Savannah and Santee River drainages, and *Micropterus calliurus* **sp. nov.** from the Altamaha and Ogeechee River drainages. Counts for each species are followed by counts for individual river systems.

	Total Scale Rows Around Caudal Peduncle							
	28	29	30	31	32	N	Mean	SD
Micropterus pucpuggy	9	9	28	14	9	69	30.07	1.18
Santee		2	6			8	29.75	0.46
Savannah	9	7	22	14	9	61	30.11	1.24
Micropterus calliurus	23	6	25	3	1	58	29.19	1.08
Altamaha	21	5	14	3	1	44	29.05	1.14
Ogeechee	2	1	11			14	29.64	0.74

Diagnosis. *Micropterus calliurus* is a monophyletic, divergent genetic lineage of bass distinguished from other species of *Micropterus* by the following combination of characters: shallow separation between the spinous- and soft-dorsal fins, glossohyal tooth patch usually present; vertically elongated lateral blotches with interspersing dorsal-lateral blotches present in juveniles and adults but often subdued; ventral-lateral rows of spots; and orange coloration on the distal portion of the first two to four soft-dorsal fin rays, distal half of the first two to five anal-fin rays, and distal one-half to one-fourth of the upper and lower caudal lobes. Other *Micropterus* species have at least one of the following: a deep notch between the spinous- and soft-dorsal fins (*M. salmoides* and *M. nigricans*; Etnier & Starnes 1993); short lateral blotches that form an irregular, lateral band (*M. punctulatus* and *M. henshalli*; Hubbs & Bailey 1940); lateral bars combined with a lack of ventral-lateral rows of spots (*M. dolomieu* and *M. velox*; Hubbs & Bailey 1940, Etnier & Starnes 1993); absence of a tooth patch (*M. cataractae* and *M. warriorensis*; Williams & Burgess 1999; Baker *et al.* 2013); and absence of chromatic color in dorsal or anal fins (*M. treculii, M. notius, M. cahabae* and *M. tallapoosae*; Bailey & Hubbs 1949; Baker et al. 2013, Robins *et al.* 2018). *Micropterus coosae* has brick-red color across the distal half of the soft-dorsal, anal and caudal fins (Baker *et al.* 2013). *Micropterus calliurus* differs from *M. chattahoochae* in having: a modally higher count of lateral-line scale rows (69–70 versus 63–66, Baker *et al.* 2013); lateral blotches that extend up to nine scale rows (versus fewer than five) below the midline; upper and lower rays and membranes of the caudal fin with salmon-orange color that does not extend around the posterior margin of the fin (versus bright orange on the upper and lower caudal rays that may wrap around the distal edge of the fin forming a marginal band); and anal fin with salmon-orange in the distal one-half of the first five inter-radial membranes (versus bright orange in the distal two-thirds of the first seven to eight inter-radial membranes). *Micropterus calliurus* differs from *M. pucpuggy* **sp. nov.** in having brighter and more consistent chromatic color on the upper and lower caudal rays and the anterior soft-dorsal fin rays (sometimes subdued or absent in *M. pucpuggy*). As noted in the diagnosis for *M. pucpuggy*, *M. calliurus* has modally lower counts of lateral-line scale rows (69–70 vs. 70–71; Table 1), scale rows above (8–9 vs. 9–10; Table 2) and below (15–16 vs. 16–18; Table 3) the lateral line,

and cheek scale rows (12–13 vs. 14–15; Table 4) than *M. pucpuggy*. *Micropterus calliurus* is also less robust than *M. pucpuggy*, having shallower relative body depth (251–300 thousandths of SL, mean 276, vs. 253–323, mean 286) and caudal peduncle depth (109–130, mean 116 vs. 113–136, mean 123; Figure 6) and a shorter relative caudal peduncle (205–241, mean 221 vs. 207–246, mean 227) and greater relative predorsal length (395–432, mean 418 vs. 386–446, mean 412; Table 5; see also diagnosis for *M. pucpuggy*).

Description. Moderately robust bass, greatest body depth of holotype 50.5 mm (286 one-thousandths of SL). Standard length to at least 248 mm SL (GMNH 2398, Oconee River, Baldwin Co, GA). Scale and fin element counts provided in Tables 1–4 and 6–10, and proportional measurements in Table 5. Modes for counts as follows: lateral-line scale rows 69–70 (most, 65–72); dorsal spines 10; dorsal rays 12; pectoral rays 15–16; anal spines 3; anal rays 10; scales above the lateral line 8–9; scales below the lateral line 15–16; caudal-peduncle scale rows 28, 30 (13–14 above and below the lateral line); cheek scale rows 12–13 The notch between the spinous- and soft-dorsal fins is shallow, with the length of shortest posterior spine averaging 72% (n=36) of the length of the longest spine. A small (sometimes scant) oval tooth patch is usually present (45 out of 56 specimens examined).

Juveniles and adults marked with nine to 11, modally 10, elongate lateral blotches that often include a vertically expanded caudal spot. Blotches extend below the lateral midline to intersect with usually seven to 10 parallel rows of spots extending from above the pelvic-fin insertion posteriorly onto the caudal peduncle. Lateral blotches extend dorsally two or three scale rows above the lateral line in anterior blotches. Dorsally, sides marked with dorsolateral, roughly oval markings that extend ventrally toward the spaces between lateral blotches, with smaller, supralateral markings between the dorsolateral ones and touching the dorsal-fin base.

Color in life. Scales above and between lateral blotches centrally light gold with olive margins. Scales below midline and between blotches light except for narrow dark centers that form parallel rows of spots. Many ventral scales from breast along sides to above anal fin occasionally have higher than wide dark pigment, and these may or not give impression of rows of spots. Dorsum of head with tan ground coloration and black dorsal and dorsolateral markings of varying width. Cheeks and head marked by three prominent stripes, first and widest in line with body axis, extending from tip of opercle through eye and immediately narrowing on snout to about nares. Next stripe narrower and extends from above angle of preopercle to eye. Third, most ventral stripe narrower and extends from about angle of preopercle to edge of maxilla. Eye red in medial posterior and anterior regions of iris, with thin gold margin around pupil. Most of upper posterior edge of iris with blue-gray membrane that extends from orbital margin to across edge of iris. Small thin blue-gray line of pigmentation adjacent to upper anterior margin of orbit present, opposite posterior pigmentation. Suborbital region about width of two scales blue-green. Posterior tip of opercular membrane silver-white.

Caudal-fin base occasionally with two basicaudal depigmented areas, above and below basicaudal spot. Caudal-fin membranes peppered with discrete light areas in otherwise pigmented membranes resulting in freckled or spotted caudal fin. Caudal-lobe tips depigmented and appear white, with leading edges of upper and lower caudal lobes salmon-orange. Anal-fin rays clear, with anterior portion of adjacent membrane pigmented to form one or more dark bordering bands of spots. Medial membranes of four or five posterior anal-fin rays with concentrations of melanophores that appear as spots, especially evident in individuals smaller than about 150 mm SL. In larger individuals anal-fin membranes appear dark. Distal half of first two to five anal-fin rays with orange color; orange color more intense in juveniles. Soft-dorsal fin basal membranes have alternating light and pigmented portions giving banded or spotted appearance. Juveniles and small adults have orange color on distal portion of first two to four soft-dorsal fin rays.

Color in alcohol. Specimens preserved in 70% ethanol lack chromatic coloration, although fin spotting remains. Lateral and dorsolateral blotches may be difficult to discern, especially in larger individuals. pores in lateral-line scales light in color, in contrast to darker scale background.

Distribution. *Micropterus calliurus* is endemic to upper Ocmulgee and Oconee watersheds of Altamaha Basin and to the headwaters of the Ogeechee basin in Georgia (Figure 1). *Micropterus calliurus* occurs primarily above the Fall Line but may be frequently collected just below the Fall Line where shoal habitats exist. As with *Micropterus pucpuggy*, pure individuals of *Micropterus calliurus* still occur throughout its historic native range, but many constituent river systems may have lost pure populations due to introgressive hybridization with *Micropterus henshalli*.

There has been some suggestion that the Ogeechee River population of the Altamaha Bass might be the result of a recent introduction. Our DAPC analysis of population structure, in fact, shows clear separation among the

Ogeechee, Oconee, and Ocmulgee systems with only minimal overlap between the Oconee and Ogeechee systems. Also notable is the occurrence of at least six Altamaha River system fish species (*Cyprinella callisema* (Jordan), *Hudsonius hudsonius* (Clinton), *Hydrophlox lutipinnis* Jordan & Brayton, *Moxostoma collapsum* (Cope), *Etheostoma hopkinsi* (Fowler), *E. inscriptum* Jordan & Brayton) in the headwaters of the Ogeechee River, where *M. calliurus* occurs. We believe this lends credence to the likelihood of faunal exchange via stream capture between the Oconee and Ogeechee that could have allowed *M. calliurus* to colonize the Ogeechee. We conclude that the native range of *M. calliurus* includes the Ogeechee River.

Etymology. The trivial epithet *calliurus* derives from the words *call -i* (G), meaning beautiful, and *urus* (G), meaning tail (Borror 1960). Treated as a noun in apposition.

Discussion

Concurrence of our 3RAD results and morphological analyses with previously published mitochondrial (Baker *et al.* 2013; Freeman *et al.* 2015) and ddRAD (Kim *et al.* 2022) phylogenies, and with biogeographic patterns of *Micropterus* diversification in southeastern rivers, support the designation of two new species of *Micropterus*, one from the Altamaha and Ogeechee River basins and one from the Savannah and Santee River basins. Here, we reflect on the emergence of the Retrospective Reproductive Community Concept (Maddison and Whitton 2023). The *Micropterus* of the southeastern United States have accreted diversity through isolation across varied riverine environments, resulting in at least 15 genetically- and phenotypically-distinct forms that are endemic to 14 separate southeastern drainage systems (Baker *et al.* 2013; Taylor *et al.* 2019; Kim *et al.* 2022). The role of black bass as apex predators in many of these rivers, their diverse phenotypes, and their interactions with biogeographically-driven communities make these evolutionarily distinct lineages "important to name" (Maddison & Whitton 2023, p. 21). The challenge is that in recognizing these species, we recognize how quickly they are becoming lost as extrinsic barriers are breached by human activities.

The two new species of *Micropterus* identified and named here are clearly distinct in an evolutionary and diagnostic sense from other species in the Black Bass clade. The timing of radiation of *M. chattahoochae* (Baker *et al.* 2013) and these two new species from the western Redeye basses is not yet clear but is likely a Pleistocene divergence (Kim *et al.* 2022) and may represent headwater capture events from the Chattahoochee into these eastern basins. Additionally, variation in lateral blotches, fin coloration, and possibly morphology suggest independent, though not necessarily adaptive, phenotypic evolution. Overall, our results are entirely concordant with the informal recognition by previous studies and applied names to these evolutionarily distinct populations of *Micropterus*. Although the phenotypic variation separating these new species is only partly quantitative–leading to difficulties for field diagnosis–the genomic separation clearly is strong and driven by persistent isolation. These isolated lineages are now being affected by introduction of *Micropterus* species from other parts of the southeast US, generating concern that this endemic diversity could be quickly lost. It is becoming more widely recognized that the process of describing novel species often must rely on genomic data and an integrative assessment of the diversity across biogeographic space (Pyron *et al.* 2023).

Conservation of *M. calliurus* and *M. pucpuggy* will depend on protecting habitat for these species and minimizing opportunities for hybridization with non-native basses. *M. calliurus* and *M. pucpuggy* are riverine species that characteristically occupy pools and runs associated with rocky shoal habitats. These species now persist in river systems where that habitat has been substantially altered by sedimentation and fragmented by dams. Beginning in the 18th century, Europeans and their descendants cleared the southern Piedmont forests to cultivate crops on land taken from displaced Indigenous North Americans, resulting in extensive soil erosion that continued into the 20th century and that filled stream and river valleys with meters of alluvium (Trimble 1974; Jackson *et al.* 2005). Piedmont streams that ran clear when Bartram saw them in the late 1770s now run muddy following rain, rocky shoals may be buried in meters of sediment, and streambed gravels are characteristically embedded with silts and sand (Trimble 1974; Jackson *et al.* 2005). European colonists and descendants also fragmented stream systems, first with dams to power mills (Walter & Merritts 2008), followed in the 19th and 20th centuries by hydroelectric dams. Nearly the entire length of the mainstem Savannah River upstream of the Fall Line has been converted to a chain of reservoirs impounded by three large hydroelectric dams, with at least eight additional hydropower dams in the Savannah River headwaters also submerging prime shoal habitats that historically supported *M. pucpuggy*.

Three large hydroelectric dams inundate and fragment the mainstem Oconee and Ocmulgee rivers, the largest rivers inhabited by *M. calliurus*. Reservoirs have in part facilitated introductions of non-native basses, of which the detrimental effects on *M. pucpuggy* are well documented (Leitner *et al.* 2015; Oswald *et al.* 2015; Bangs *et al.* 2018). Non-native *M. henshalli* have replaced or introgressed with *M. pucpuggy* in reservoirs of the upper Savannah system (Barwick *et al.* 2006, Bangs *et al.* 2018). *Micropterus pucpuggy* collected from Savannah system tributaries also show high rates of introgression with non-natives (mostly *M. henshalli*), with genetically pure individuals occurring more commonly in the Blue Ridge headwaters than in the Piedmont and at locations farther from reservoirs (Judson *et al.* 2021, Peoples *et al.* 2021, Cox 2022). *Micropterus calliurus* appears similarly threatened by introduced *M. henshalli* in the Oconee and Ocmulgee systems, although quantitative field studies could help identify locations in the native range where pure populations are most likely to persist.

Introgression with non-native basses has made it challenging to assemble type series to assign species names to *M. calliurus* and *M. pucpuggy*; conserving the unique genetic diversity inherent in each of these species will be even more challenging. The high resolution available from thousands of SNPs has allowed us to base formal species descriptions of *M. calliurus* and *M. pucpuggy* on non-introgressed individuals identified with reasonably high confidence. Genome-wide sequencing may provide an important tool for identifying shifts in degrees and frequency of introgression with non-native basses in response to future management aimed at conserving *M. calliurus* and *M. pucpuggy*. Although recent hybrids between either of these native taxa and non-native *M. henshalli* or *M. dolomieu* can be readily identified genetically and sometimes visually (Figure 5F, e.g., showing lateral blotches merged into a lateral band, typical of *M. henshalli*), field identification of hybridized *M. pucpuggy* and *M. henshalli* can be difficult if the percent introgression is low. Back-crossed individuals may display lateral pigmentation and even fin color characteristic of the native species (Figure 5G, e.g.). Translocation or population augmentation efforts intended to reestablish pure populations should include genetic confirmation of the integrity of stocked or translocated individuals. Such efforts might also aim to conserve the fine-scale genetic differences among isolated populations of *M. pucpuggy* and *M. calliurus* as evidenced by mitochondrial sequences (for *M. pucpuggy*; Oswald *et al.* 2015) and our 3RAD analyses. Habitat restoration efforts involving instream barrier removal may also require careful consideration of whether specific barriers lower population resilience (e.g., by limiting recolonization following catastrophic events) or, conversely, protect native bass populations from stocked, invasive bass species. Fishery managers may need a robust plan informed by genomic studies as recovery and management of these two taxa proceed.

Non-type Tissue Samples

Micropterus calliurus, Georgia, Monroe County, Tobesofkee Creek (32.9936, -83.9439), 3 individuals (GMNHTC 132.22.36, 132.22.37, 132.22.38), 12 October 2022, B. Bowen, GNDR; *Micropterus calliurus,* Georgia, Warren County, Ogeechee River (33.2955, -83.7812), 5 individuals (GMNHTC 70.21.45, 70.21.48–70.21.50, 71.21.51), 30 September 2021, B. Bowen, GNDR; *Micropterus pucpuggy*, South Carolina, Laurens County, Enoree River (34.6493, -81.9591), 1 individual (GMNHTC 16630), 15 November 2010, J. Leitner, SCDNR; *Micropterus chattahoochae*, Georgia, White County, Chattahoochee River at Nora Mill, 2.3 air km SE Helen, GA city center (34.6902, -83.7112), 1 individual (GMNHTC 10390), 23 September 2010, B.J. Freeman, M.M. Hagler, C.A. Straight; *Micropterus chattahoochae*, Georgia, White County, Chattahoochee River North of Helen, alongside GA Hwy 75 (34.7226, -83.7479), 2 individuals (GMNHTC 12440, 12441), 1 December 2015, B. Albanese, GDNR, J. Tomelleri; *Micropterus chattahoochae*, Puerto Rico, Rio Rosario at Vivero de Maricao, on river above dam (18.1656, -66.9882), 1 individual (GMNHTC 12128), 18 April 2013, B.J. Freeman, M.C. Freeman, Samuel Garcia; *Micropterus coosae*, Georgia, Gilmer County, Mountaintown Creek upstream of SR 282, 5.1 air km W. of Elijay (34.7051, -84.5392), 2 individuals (GMNHTC 2112, 2116), 7 October 2004, P.A, Marcinek, GDNR; *Micropterus coosae*, Georgia, Murray-White Counties, Conasauga River, ca. 1.0 river km downstream from mouth of Jacks River, 7.9 air km ESE of Conasauga, TN city center (34.9960, -84.6433), 1 individual (GMNHTC 3909), 18 September 2001, B.J. Freeman; *Micropterus coosae*, Georgia, Dawson County, Russell Creek 0.5 river km upstream of County Road 76 (Stowers Rd.), 6.3 air km SE of Dawsonville, GA city Center (34.3985, -84.0566), 1 individual (GMNHTC 5951), 11 May 2005, B.J. Freeman, C. Storey, M.M. Hagler; *Micropterus coosae*, Alabama, Cherokee County, Terrapin Creek along State Route 9, 8.2 air km NW of Spring Garden, AL city center (34.0276, -85.6138),

1 individual (GMNHTC 3953), 19 October 2001, B.A. Porter, C.M. Storey, C.A. Straight, J.R. Knight; *Micropterus henshalli*, Georgia, Cherokee County, Etowah River at County Route 782 (East Cherokee Drive), 4.7 air km SSW of Ball Ground, GA city center (34.2992, -84.3965), 1 individual (GMNHTC 7370), 8 September 2001, B. J. Freeman, M.M. Hagler, B.A. Porter, E.M. Orms, J.R. Knight, M.C. Freeman, J.C. Shields; *Micropterus henshalli*, Georgia, Murray-White Counties, Conasauga River at shoal immediately upstream of U.S. Highway 76 / State Route 52, 7.8 air km W of Chatsworth, GA, city center. (34.7844, -84.8731), 1 individual (GMNHTC 7378), 17 September 2002, B.A. Porter, J.C. Shields, N.M. Burkhead, B.L. Nuese, E.A. Curry, D.H. Huge; *Micropterus henshalli*, Georgia, Cherokee County, Little River at County Route 388 (Trickum Road), 4.5 air km E of Woodstock, GA city center (34.1036, -84.4704), 2 individuals (GMNHTC 8646, 8647), 17 October 2001, J.R. Knight, GDNR; *Micropterus dolomieu,* Canada, Ontario, Lakes near Fort Frances (48.62, -93.42), 2 individuals (GMNHTC 3680, 3697), 7 August 2005, T. Reinert; *Micropterus dolomieu,* North Carolina, Swain County, Little Tennessee River along Lower Needmore Road (35.3267, -83.5240), 3 individuals (GMNHTC 8649, 8650, 8651), 27 June 2000, B.A. Porter, B. McCoy, D.M Walters; *Micropterus dolomieu,* South Carolina, Edgefield County, Savannah River at Augusta Shoals (33.4947, -81.9908), 1 individual (GMNTC 16671), 25 May 2023, B.J. Freeman, K. Kuback, D. Gelder, R. Moore, K. Lusk, SCDNR; *Micropterus cataractae*, Georgia, Hall County, Chattahoochee River upstream of Mud Creek (34.4729, -83.6873), 4 individuals (GMNHTC 3525, 3531, 3533, 3537), 10 September 2005, B.J. Freeman, C.A. Straight; *Micropterus salmoides*, Georgia, Carroll County, Little Tallapoosa River at County Route 828 (Farmers High Road), 5.0 air km SE of Bowdon, GA city center (33.5105, -85.2106), 1 individual (GMNHTC 8645), 25 May 2002, M.C. Freeman, M.M. Hagler, J.C. Shields, R.O. Crehan, K.C. Dee.

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Contributions

B.J. Freeman began this project and was responsible for acquisition of specimens, photography, extraction of gDNA, archiving tissues and specimens, counts and measurements, 3RAD analysis and manuscript preparation. M.C. Scott facilitated funding, provided specimens, project guidance and input on data interpretation. K. Petersen prepared libraries and assembled the entire 3RAD dataset for downstream analysis. N. Bayona-Vásquez prepared libraries and consulted on 3RAD and genetic analyses. A.T. Taylor contributed count and measurement data. B.G. Hilburn assisted with fieldwork, database work, molecular bench work and preparation of the distributional map and final manuscript. M.C. Freeman contributed meristic and morphological measurements and analyses and prepared the final draft manuscript. J.P. Wares was responsible for the bio-informatic computational framework and analysis. All authors contributed edits to the final submitted manuscript.

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Supplementary Materials. The following supporting information, tables and figures are available for download from FigShare: https://doi.org/10.6084/m9.figshare.29611040

Supplement 1. Collection localities for *Micropterus pucpuggy* and *Micropterus calliurus*, listing the number of individuals from each locality included in the type series for each taxon, the number of individuals represented by tissue- or DNA-only, the number of type specimens represented in the phylogenetic analysis, and the number of types or tissue-only samples used on the population structure analysis (Discriminant Analysis of Principal Components, DAPC). A dash (–) indicates no specimens.

Supplement 2. Parameter choice for STACKS

Supplement 3. Collection and GA Museum of Natural History Tissue collection (GMNHTC) data for 171 specimens, including types for *Micropterus pucpuggy* and *Micropterus calliurus*, and materials used for population comparisons and for phylogenetic analyses, with 3RAD sequence reads accessioned at NCMI BioProject PRJNA1152248.