Noturus munitus, Buttahatchee R., Lowndes Co., AL

Final Report: Status review and surveys for Frecklebelly Madtom, *Noturus munitus*.

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Background:

The frecklebelly madtom, *Noturus munitus*, was described by Suttkus & Taylor (1965) based largely on specimens from the Pearl River in Louisiana and Mississippi. At that time, the only other known populations occupied the Cahaba and Upper Tombigbee rivers in Alabama and Mississippi. Populations were subsequently discovered in the Alabama, Etowah, and Conasauga rivers (Bryant et. al., 1979).

The disjunct distribution displayed by the species prompted an examination of morphological and genetic differentiation between populations of frecklebelly madtom. Between 1997-2001 I gathered morphological, meristic, and mtDNA sequence data on frecklebelly madtoms from across their range. Preliminary morphological data suggested that while there was considerable morphological variation across the range, the populations in the Coosa River drainage above the Fall Line were the most distinctive population and were diagnosable as a distinct form. I presented a talk on this at the Association of Southeastern Biologists meeting in 1998, and despite the lack of a formal description, the published abstract referring to a "Coosa Madtom" made it into the public

eye (Neely et al 1998, Boschung and Mayden 2004). This has resulted in substantial confusion over conservation priorities and the status of this form.

The subsequent (and also unpublished) mitochondrial DNA data set, however, suggested that populations were moderately differentiated, shared no haplotypes and were related to one another in the following pattern: Pearl[Tombigbee[Cahaba+Coosa]]. I withheld submitting the manuscript with hopes of adding more genetic data and increasing the sample size of the morphological data set, neither of which have occurred. I have reanalyzed both the existing morphological and mtDNA data sets, treated herein, to make this preliminary data set available to the conservation community. I would caution that additional data sets might provide different perspectives on variation in this species; and further suggest that a microsatellite or SNP data set could be extremely informative in assessing genetic diversity within this madtom.

Methods.

Institutional abbreviations generally follow Leviton et al. (1985) and Leviton & Gibbs (1988).

Morphology:

Morphometrics. I used a modified truss system (sensu Strauss & Bookstein, 1982; Bookstein et al., 1985) composed of distances between 12 landmarks representing the lateral shape of the specimen, 3 distances between 6 landmarks related to the dorsal head shape, and several miscellaneous measurements (Fig. 1). Measurements were recorded to the nearest 0.1mm, using needle-point calipers under magnification. No individuals under 30 mm SL were included in this analysis because of the potential for undeveloped lateral line pores and allometric errors (Blaxter, 1987). Madtoms in the subgenus *Rabida* are prone to distortion upon preservation in formalin; as many as 60-80% of available specimens have the posterior part of the body deflected ventrally at an acute angle (DAN, pers. obs). Specimens displaying substantial ventral deflection of the caudal peduncle were excluded from morphometric analyses.

Measurements and acronyms (in parentheses) are given below and depicted in Fig. 1. Midsagittal measurements included: (SNNP) tip of snout to nape [posterior edge of the supraoccipital], (NPDO) nape to dorsal fin origin, (DOADO) dorsal fin origin to adipose fin origin, (ADOADI) adipose fin origin to adipose fin insertion, (ADIDH) adipose fin insertion to dorsoposterior edge of hypural plate, (DHVH) dorsoposterior edge of hypural plate to ventroposterior edge of hypural plate, (VHAI) ventroposterior margin of hypural plate to anal fin insertion, (AIAO) anal fin insertion to anal fin origin , (AOPV) anal fin origin to midpoint between pectoral fin origins, (PVBR) midpoint between pectoral fin origins to isthmus of branchiostegal membranes, (BRBA) isthmus of branchiostegal membranes to midpoint between middle pair of mental barbels, (SNBA) midpoint between bases of middle pair of mental barbels to tip of snout, (SNITH) tip of snout to isthmus of branchiostegal membranes, (BANP) midpoint between middle pair of mental barbels to nape, (BRNP) nape to isthmus of branchiostegal membranes, (BRDO) isthmus of

branchiostegal membranes to dorsal fin origin, (NPPV) nape to midpoint between pectoral fin origins, (PVDO) midpoint between pectoral fin origins to dorsal fin origin, (DOAO) dorsal fin origin to anal fin origin, (PVADO) midpoint between pectoral fin origins to adipose fin origin, (ADOAO) adipose fin origin to anal fin origin, (ADOAI) adipose fin origin to anal fin insertion, (AOADI) anal fin origin to adipose fin insertion, (ADIAI) anal fin insertion to adipose fin insertion, (AIDH) anal fin insertion to dorsoposterior edge of hypural plate, (ADIVH) adipose fin insertion to ventroposterior edge of the hypural plate. Dorsal cephalic measurements included: (GAPE) gape width, (IOW) interorbital width [including fleshy tissue around rim of orbit], (BW) body width [taken from bases of the pectoral spines]. Other measurements taken included: (ORB) orbit diameter [in horizontal plane, including fleshy tissue around rim of orbit], (CDRAY) longest caudal ray, (ADH) maximum height of the adipose fin, (ADBH) maximum height of the adipose bar, (SD2L) length of saddle II along dorsal midline, (SD3L) length of saddle III along dorsal midline (along crease between body and adipose fin).

An initial analysis suggested that the characters associated with dorsal saddles and the adipose bar "swamped out" other shape variables; a second analysis was conducted with those variables (ADBH, SD2L, SD3L) excluded.

These data were analyzed with principal components analysis (PCA) (SAS for Windows, Cary, NC 1996) using the covariance matrix of log-transformed measurements. We used the shearing program of Swofford (unpublished, 1984, privately distributed) as modified by M.L. Warren (unpublished, 1993) to remove the effects of size from all comparisons. This technique allows estimation of actual shape differences between populations. MANOVA was used to test whether population clusters were significantly different ($P<0.05$). Analyses were conducted separately on each sex and then pooled if no significant differences were observed.

Meristics. We generally followed methodologies of Hubbs & Lagler (1964), except for the number of serrae on the anterior and posterior sides of the pectoral spine, which was adopted from Taylor (1969). The lateral line in almost all specimens was highly interrupted on the posterior half of the body; we included disjunct pores along the lateral series to the posterior margin of the hypural plate in the total count. The fleshy tissue covering the base of the anal fin often required both making an incision and using transillumination to accurately enumerate rudimentary rays at the fin origin.

Pigmentation characters. Specimens from populations representing the entire geographic range of *Noturus munitus* were examined to identify within- and between-population variation in pigmentation characters. We generally followed Taylor (1969) in nomenclature, but due to inconsistencies with saddle descriptions, we used our own numbering scheme to refer to the saddles and ground pigmentation between the saddles (intersaddles). Saddle I is located under the dorsal fin, saddle II midway between the dorsal and adipose fin, saddle III under the adipose fin (extends dorsally onto the fin to become the adipose bar), and saddle IV is on the caudal peduncle. We measured the length of saddles II and III along the dorsal midline in each specimen used for the morphometric analysis. Life colors were recorded from small lots from each occupied drainage (Coosa,

Cahaba, Tombigbee, and Pearl) both after capture and intermittently during holding in aquaria. These specimens were maintained for roughly 1 yr.

Genetic:

Frecklebelly Madtoms were collected from across their range using either seining or electrofishing. Whole specimens or tissue samples for genetic analyses were either frozen or fixed in 95% EtOH. Voucher specimens for most samples are catalogued at the University of Alabama Ichthyological Collection, Tuscaloosa, Alabama. DNA was extracted from tissue samples using DNEasy kits (QIAGEN, Valencia, CA). Genomic extract was used as template to amplify the complete mitochondrial cytochrome b and ND2 genes via PCR. Primers included L14724, 5'-gtgacttgaaaaaccaccgttg-3', (Schmidt & Gold 1993) and H15915, 5'-ctccatctccggtttacaagac-3' (Song et al. 1998). Each 25 µL reaction contained 1 µL genomic DNA, each primer at 1.0 mM, 4.0 µM MgCl2, 2.5 µL 10x reaction buffer, and 1.25 units of Taq polymerase. Amplifications consisted of 35 cycles, each with 45 sec. denaturation at 94ºC, 45 sec. annealing at 50ºC, and 60 sec. extension at 70ºC. PCR products were purified of unincorporated primers and nucleotides with QIAGEN Quick-Clean kits (QIAGEN, Valencia, CA). Sequencing reactions used a dye-labeled dideoxy terminator sequencing kit (Big Dye) and were visualized on an ABI 3100 automated sequencer (University of Alabama, Tuscaloosa). Both light and heavy strands were sequenced for all samples.

Sequences were verified by consensus between the two strands, edited, and aligned by eye using BioEdit v5.0.9 (Hall, 2001). Veracity of all mutations was assessed via comparative alignment and examination of the electropherograms using BioEdit.

Parsimony analysis (MP) utilized the heuristic search option in PAUP* v4.0a164, employing accelerated transformation, tree-bisection-reconnection, and random sequence addition. MP analyses were conducted with molecular characters unweighted and unordered. All minimal-length trees were kept, and zero-length branches collapsed. Support for individual nodes was assessed both by performing 1000 jackknife replicates with 37% data deletion in each replicate and JAC emulation selected, and 1000 bootstrap replicates using 50% resampling.

Outgroup taxa included several additional members of the genus *Noturus*, as suggested by the analyses of Hardman (2003). These included *Noturus gladiator, N. stigmosus, N. eleutherus, N. flavater, N. furiosus,* and *N. placidus*.

Materials Examined: Numbers in parentheses refer to the number of specimens measured and the number of specimens in the lot.

Morphometrics:
Pearl River: TU 110576 Pearl River, Lawrence Co., MS (20/153). Tombigbee River: UAIC 4420.21 Tombigbee River, Lowndes Co., MS (1/2); UAIC 4389 Tombigbee River, Lowndes Co., MS (3); UAIC 4388 Tombigbee River, Lowndes Co., MS (1); UAIC 4387 Tombigbee River, Lowndes Co., MS (1/3) UAIC 4393 Tombigbee River, Pickens Co., AL (1); UAIC 5729

Sipsey River, Tuscaloosa Co., AL (2); UAIC 6461 Bull Mountain Creek, Itawamba Co., MS (3); UAIC 9570 Tombigbee River, Monroe Co., MS (1/16); UAIC 7171.01 Sipsey River, Tuscaloosa Co., AL (1); UAIC 8587.01 Buttahatchee River, Monroe/Lowndes Co., AL (3/6); UAIC 11874.01 Buttahatchee River, Monroe/Lowndes Co., AL (2/3). Cahaba River: UAIC 6780.15 Cahaba River, Perry Co., AL (2); UAIC 5819.07 Cahaba River, Perry Co., AL (1); UAIC 9584.13 Cahaba River, Bibb Co., AL (8/10); UAIC 9592.20 Cahaba River, Perry Co., AL (1/5); UAIC 9607.13 Cahaba River, Perry Co., AL (1/9); UAIC 10510.17 Cahaba River, Bibb Co., AL (1);UAIC 10511.12 Cahaba River, Bibb Co., AL (1); UAIC 11701.01 Cahaba River, Perry Co., AL (4/10). Etowah River: UAIC 8862.01 Etowah River, Cherokee Co., GA (3/3); UAIC 6217.01 Etowah River, Dawson Co., GA (1/1); UAIC 10624.09 Etowah River, Dawson Co., GA (5/5)

Additional material examined for meristics and coloration:
Pearl River: NLU 11916 Strong River, Simpson Co., MS (135); NLU 29168 Boguechitto River, Washington Co., LA (139). Tombigbee River: UAIC 3096, Tombigbee River, Greene Co., AL (10). UAIC 4331.12 Tombigbee River, Pickens Co., AL (49); UAIC 4750.16 Tombigbee River, Lowndes Co., MS (1); UAIC 4430.17 Tombigbee River, Monroe Co., MS (1); UAIC 4425.19 Tombigbee River, Lowndes Co., MS (10); UAIC 2593 Tombigbee River, Pickens Co., AL (3); UAIC 6477.01 Sipsey Creek, Monroe Co., MS (4); UAIC 4418.09 Tombigbee River, Lowndes Co., MS (1); UAIC 4750.16 Tombigbee River, Pickens Co., AL (6); UAIC 6478.01 Sipsey Creek, Monroe Co., MS (1); UAIC 4337.10 Tombigbee River, Pickens Co., AL (18); UAIC 2705 Tombigbee River, Pickens Co., AL (25); UAIC 4440.18 Tombigbee River, Monroe Co., MS (1); UAIC 4338.20 Tombigbee River, Lowndes Co., MS (12); UAIC 4402.26 Tombigbee River, Lowndes Co., MS (8); UAIC 4330.19 Tombigbee River, Pickens Co., AL (22); UAIC 1470 Tombigbee, Sumter-Greene Cos., AL (1). Cahaba River: UAIC 1437 Cahaba River, Perry Co., AL (2); UAIC 6782.10 Cahaba River, Perry Co., AL (1); UAIC 6797.16 Cahaba River, Bibb Co., AL (2); UAIC 7186.17 Cahaba River, Bibb Co., AL (16); UAIC 7191.16 Cahaba River, Perry Co., AL (5 c&s/6); UAIC 9607.13, Cahaba River, Bibb Co., AL (9/9); UAIC 11701.01 Cahaba River, Bibb Co., AL (1). Coosa River: UGAMNH 1598 Etowah River, Cherokee Co., GA (6); UGAMNH 3001 Etowah River, Dawson Co., GA (1); UGAMNH 2323 Etowah River, Dawson Co., GA (10); UGAMNH 2326 Etowah River, Dawson Co., GA (8); UGAMNH 2448 Etowah River, Dawson Co., GA (7); UGAMNH 2452 Etowah River, Dawson Co., GA (5/6); UGAMNH 2633 Etowah River, Cherokee Co., GA (2); UGAMNH 1620 Conasauga River, Murray-Whitfield Cos., GA (1); UGAMNH 1622 Conasauga River, Murray-Whitfield Cos., GA (2); UAIC 10487 Conasauga River, Murray-Whitfield Cos., GA (3/4). UAIC 3901.02 Conasauga River, Bradley Co., TN (1). UF 86146, Etowah River, Cherokee Co., GA (11); UF 86209, Etowah River, Dawson Co., GA (12); UF 86232, Etowah River, Dawson Co., GA (11); UF 86248, Etowah River, Forsyth Co., GA (15).

Genetics:
Pearl River: UAIC 12758 LA:St. Tammany:Pushepatapa Cr (3). UAIC 12671 MS:Marion:Pearl R (5). Tombigbee River: UAIC 11874.01 AL:Marion:Buttahatchee R (5). Cahaba River: UAIC 11701.01 AL:Bibb:Cahaba R (5). UAIC 11960.01 AL:Bibb:Cahaba R (2). UAIC 12702.10 AL:Perry:Cahaba R (1). Etowah River: UAIC 12452.02 GA:Dawson:Etowah R (2). UAIC 12453.07 GA:Cherokee:Etowah R (1).

Results

***Morphological variation*:**
Body shape variation.--An initial sheared principal component analysis (conducted in 1998) identified substantial differentiation between populations in both males(Fig. 2) and females (Fig. 3), based primarily on two variables; the height of the adipose fin bar (ADBH), and the length of saddles II and III (SD2L, SD3L)(Tables 1, 2). At the time, the degree of difference observed between Coosa populations and the other drainages examined appeared to mirror the situation in the upland *Noturus stigmosus*- lowland *Noturus gladiator* pair (*N. gladiator* was not formally described until 2004; Thomas and Burr 2004). This is in large part what led to the ASB presentation suggesting that the Coosa River populations were distinct. However, it is important to note that while the means are significantly different, there is a considerable degree of overlap between all of the populations and neither the adipose bar height nor the length of saddles II and III are completely diagnostic for the Coosa populations (Table 3).

When these variables were excluded from the analysis, several other variables suggest some body shape differentiation among populations in both males (Fig 4., Table 4) and females (Fig. 5, Table 5), although there was considerable overlap of minimum-spanning polygons in all analyses. The only consistent patterns across sexes were that the snout-barbel midpoint distance (SNBA) was longer in the Pearl and shortest in the Coosa madtoms, and that that the distance between the rear of the adipose fin and the dorsal end of the caudal peduncle (ADIDH) was longer in the Cahaba, and shorter in other populations (Table 6). However, these differences are not diagnostic.

Sexual Dimorphism.--Males exhibited swelling of the cephalic epaxial musculature, lips, and genital papilla during the mid-summer breeding season. This trait is widespread among various species of *Noturus* (Taylor 1969). No other differences were noted between the sexes.

Meristics.--No significant differences were observed between populations (Table 7).

Color and pigmentation:
Specimens from the Pearl River drainage were consistently more boldly patterned than other populations. Specimens from the Tombigbee River drainage were often (but not always) darker overall. Specimens from the Coosa river drainage had an adipose fin bar (a dorsal extension of Saddle III extending onto the adipose fin) that was usually slanted posterodorsad, and usually restricted to the basal 55 - 84% (x = 80%) of the fin. In all Pearl River material examined, and most specimens from the Tombigbee and Cahaba, the bar is not slanted, but instead oriented vertically, and reaching the posterior margin of the adipose fin.

Color in preservative.--Dorsal saddles dark brown to blackish, first saddle centered under spine and first three rays of dorsal fin; second saddle from directly above pelvic fin origin to adipose fin origin; third saddle below adipose fin and extending dorsad onto the fin, forming an adipose bar with vertically-aligned lateral margins and which reaches the distal

margin of fin; fourth saddle crossing caudal peduncle from third or fourth procurrent caudal-fin ray posteriorly to base of caudal fin. First saddle extends ventrally to well below pectoral fin insertion, becoming stippled ventrally. Dusky nuchal bar midway between nape and base of dorsal spine partially or completely enclosing two pale areas, one on either side of the dorsal midline immediately anterior to the base of dorsal spine. Sides mottled with greys, yellow-ochre, and browns. Intersaddles unmarked, light yellowish brown to yellowish grey. Area above posterior extension of cleithrum light yellowish-brown. Pale spot of same diameter or larger than eye immediately posterior to orbit. Top of head dusky-brownish, darker towards nape, and in area immediately anterior to orbit. Lips pale, stippled or freckled. Pale sulphur-yellow colored area immediately ventral to orbit, at angle of jaws. Anal fin cream to whitish at base, yellowish distally, and with two dusky bands crossing fin. Pelvic fin yellowish, with medial dusky flecks. Pectoral fin pale basally, yellowish distally, and with subdistal black band; tissue covering spine is dusky. Dorsal fin sulphur-yellow, black basally, with two or three bands crossing fin, subdistal band most intense; tissue covering spine dusky. Adipose fin yellow anterior of bar, clear posteriorly. Blackish mid-caudal bar crescent-shaped, enclosing a pair of lunate yellow areas on the basal part of the caudal fin. Two dusky-black bars posteriorly on caudal fin, and a translucent band on distal margin of fin. Intersaddles golden yellow, with faint orangish undertones. Belly cream-white, with small, widely scattered melanophores. Mental barbels with scattered blotches, generally pale cream. Maxillary barbels dusky basally, pale distally.

Large larval specimens of *Noturus munitus* from the Tombigbee (UAIC 4402.26, 14.4 mm SL) and Cahaba drainages (UAIC 9607.13, 14.5 mm SL) exhibited moderate to strong saddle development. The Tombigbee specimen, from July, possessed small to moderate-sized, widely scattered melanophores. The Cahaba specimen, though smaller, was collected in early September, and had well developed saddles and large, squarish melanophores. A 20 mm SL specimen (UF 86248, 19 September 1990) from the Etowah had large squarish melanophores along the ventrolateral surface, dorsum with saddle pattern well developed, dorsolateral surface generally evenly pigmented, melanophores extending onto breast.

Color in life.--Color notes of *Noturus munitus* were taken from UAIC 11960.01, a 57 mm SL female from the Cahaba River, Bibb Co., AL, as well as additional specimens from the same locality maintained in aquaria for ca. 1 yr; UAIC 10487.06, a 55 mm SL female from the Etowah River, Cherokee Co., GA, as well as UAIC 12452.02, six adult specimens from the same locality maintained alive in aquaria for ca. 1 yr. Aquarium specimens adjusted pigment intensity depending on substrate color (over a 24-48 hr period), but patterns did not change.

Dorsal saddles dark brown to blackish. Sides yellow-brown, with fine dusky or black stippling. Nuchal bar usually completely encloses two pale yellow-brown areas. Area between pectoral fin base and posterior extension of the cleithrum light yellowish-brown. Light yellow-olive spot roughly same diameter as eye immediately posterior to orbit. Top of head dusky-brownish, darker towards nape and in area immediately anterior to orbit. Suborbital area sulfur-yellow, brightest at angle of jaws. Anal fin yellowish, with two dusky bands crossing fin, and a milky-clear distal margin. Pelvic fin yellowish, with medial dusky

flecks. Pectoral fin pale basally, yellowish distally, with subdistal black band. Tissue covering spine is dusky. Dorsal fin sulphur yellow, black basally, spine dusky, with two or three bands crossing fin, distal-most two bands somewhat fused to suggest broad band. Adipose fin yellow-brown anterior of bar, yellow-clear posteriorly. Blackish mid-caudal-fin bar is crescent-shaped, enclosing a pair of lunate yellow areas on the basal part of the caudal fin. Caudal fin with two other dusky-black bars posteriorly and a milky-translucent band on distal margin of fin. Intersaddles golden-yellow to yellow-brown, with faint orangish undertones. Belly cream-white, with scattered melanophores.

Genetic variation

Interpopulation genetic variation.--Considerable variation was identified between populations of *Noturus munitus*. Populations from the Pearl River drainage differed from populations in the Mobile Basin by 1.1-1.6% sequence divergence (Table 8). Within the Mobile Basin, populations from the Tombigbee River drainage differed from populations in the Alabama River drainage (Coosa and Cahaba drainages) by 0.9-1.1%. Samples from the Cahaba River differed from Etowah River *N. munitus* by 0.2-0.3%.

Intrapopulation genetic variation.--No variation was observed in specimens from the Etowah (n=3) or Pearl (n=9) drainages. Within the Cahaba (n= 12), 2 haplotypes were observed, with uncorrected p-distances ranging from 0-0.0009. Within the Tombigbee (n=5), 4 haplotypes were observed, with uncorrected p-distances ranging from 0-0.003 (Table 8).

Phylogenetic relationships.--Of the 1130 characters, 122 were phylogenetically infomative, while an additional 59 were variable but not phylogenetically informative. Parsimony analysis recovered six equally parsimonius trees of length 211, which differ principally in minor rearrangements in the trichotomy between the Pearl, Tombigbee, and Coosa + Cahaba clades. One of these trees is presented as a phylogram in Fig. 6. All analyses recovered a monophyletic clade of *Noturus munitus* populations, with strong support (98 bootstrap, 100 jackknife; Fig. 7). Within the Mobile Basin, populations from the Cahaba River were recovered as sister to the Etowah River samples in all analyses (100 bootstrap, 98 jackknife, Fig. 7).

Discussion

The moderate degree of differentiation between the Pearl River and Mobile Basin, and further between the Tombigbee and Alabama River systems, is consistent with several other aquatic organisms. In some cases these lineages have been formally described (*Percina aurora* + *P. brevicauda*, Suttkus et al. 2004, Near 2002; *Notropis ammophilus* + *N. longirostris* Raley and Wood 2001), in others, cryptic taxa appear to be involved (*Potamilis inflata*, Roe 1999; *Crystallaria asprella*, Wood et al. 2000, Morrison et al. 2006; etc.). Several taxa (*Cycleptus meridionalis, Percina lenticula*) are shared between the Pearl and Mobile drainages. For some of these sequence data are available -- the *Percina aurora*/*brevicauda* (AF386566 vs. AF386567) pair differ by 1.4% uncorrected sequence divergence at cyt *b*, between the Pascagoula and the Mobile Basin. *Notropis ammophilus* (MG806604) and *N.*

longirostris (MG806614) differ by 2.0% at cyt *b*. *Percina lenticula* from the Cahaba (JN028018) differs from the Pascagoula (Leaf R) (KF930250) by 1.5%.

The level of genetic differentiation observed between populations of *Noturus munitus* is greater than the *Noturus stigmosus/Noturus gladiator* pair, which is the sister group to *Noturus munitus*. However, in that group, morphological and genetic patterns of differentiation follow a largely congruent pattern, whereas in *N. munitus*, the weak patterns of morphological variation observed are incongruent with the genetic data, and at this time do not allow clear diagnosis of distinct species within *Noturus munitus*. This, of course, could change with different data sets or additional genetic data.

I suggest that minimally, each of these populations should be managed as a distinct ESU (*sensu* Waples 2000).

Zoogeographic implications:

The shared ~1.5% level of differentiation shared between Pearl/Pascagoula and Mobile Basin populations of *Noturus munitus, Percina aurora/brevicauda*, and *Percina lenticula* suggest at least 1.5 Mya of separation, which would place the most recent connection between the drainages in the early Pleistocene. Pre-Pleistocene habitats in the region included extensive portions of the Continental Shelf that are now inundated by the Gulf of Mexico. The connection between these systems may have occurred in this region. This hypothesis is not novel (Swift et al. 1986), and has been suggested previously by Thornberry (1965) who presented data on faunal similarities between these systems in favor of such a transfer. Alternatively, Fitzpatrick (1989) suggested that Blue Ridge erratics in north-central Mississippi supported a Pliocene track of the Old Tennessee River that was considerably further south and west than other hypotheses. This drainage would have entered the Gulf of Mexico in the vicinity of the Pearl River delta.

I suggest the sister-taxon relationship between *N. munitus* and the *N. stigmosus/N. gladiator* pair supports Thornberry's hypothesis, as does the absence of a representative of the *N. stigmosus* clade from the Tennessee River system, and presence of *N. gladiator* in lower Mississippi River tributaries.

Ecology and life history:

Based on development of mature oocytes in females, *Noturus munitus* presumably spawns in June and July in the Etowah River. Four females at peak reproductive development (two each from 30 June and 20 July) were available for study. The number of mature ova ranged from 43-88 (x=74.8); of 60 randomly selected mature ova, diameters ranged from 1.0 mm to 2.4 mm (x=1.8 mm). This is similar to *Noturus munitus* from the Tombigbee (Trauth et al. 1981; females averaged 100-140 mature ova of ca. 2 mm diameter) and Cahaba (Bennett et al. 2010), and generally similar to other members of the *Noturus flavipinnis* clade (Grady 1992, Hardman 2004).

Length-frequency analysis of 54 specimens of *N. munitus* from the Etowah River (Fig. 13) and 206 specimens from the Tombigbee River (Fig. 14) suggests the presence of four age classes in each system. Based on the smallest gravid females observed, it appears that sexual maturity is not reached until age two, at around 45-50 mm SL. This is also similar to *Noturus munitus* from the Cahaba (Bennett et al. 2010).

Conservation Status:

The frecklebelly madtom has long been given protected status in parts of its range (Ramsey 1986; Warren et al. 1997, 2000). Populations in individual drainage basins have somewhat differing life history patterns (Trauth et al. 1981; Miller 1984; Bennett et al. 2010; DAN, unpubl. data), and thus deserve mention separately.

The Pearl River population occupies both the mainstem Pearl and multiple tributaries (Suttkus and Taylor 1965, Ross 2000). Piller et al. (2004) suggested that the Pearl River population declined precipitously following channel and flow modifications on the mainstem and was most abundant in a few large tributaries. In October 2000, Steve Powers, Nick Lang, and I collected the Pearl River at the type locality and found *N. munitus* to be incredibly abundant; we observed well over 100 individuals. In contrast to the bleak outlook given by Piller et al. (2004), recent surveys (Matt Wagner, pers. comm. 2018) have found *N. munitus* to be relatively abundant over most of their historical range in the Pearl drainage.

Channel modifications associated with the construction of the Tennessee-Tombigbee waterway in the mid-1980s eliminated this species from the lower mainstem Tombigbee River where (based on pre-impoundment surveys) it was once rather common. The Tombigbee River population is now restricted to a short reach of the upper mainstem and a handful of tributary systems; Buttahatchie River, Luxapillila Creek, and Sipsey River (Shepard et. al., 1997; Ross 2000; Bennett et al. 2008). The Sipsey River is a particularly interesting system; though there are only a handful of verified records, access is poor -- and as much of the middle part of the system is a large wetland area, riffle density is low and water levels often preclude effective sampling. I suspect that *N. munitus* still occupies much of the system,but is very patchily distributed through the system. Future surveys are planned.

The mainstem Cahaba River represents a stronghold for the species (as currently recognized), and abundances are high in the mainstem from the Fall Line at Centreville downstream approximately 60 miles (Bennett et al. 2008, 2010; this study). However, this population is nearly a single linear population, does not appear to routinely use tributaries to the extent that the Pearl and Tombigbee populations do, and is thus particularly vulnerable to a catastrophic spill or other single event.

The mainstem Alabama River population was restricted to clean, stable gravel bars, a habitat mostly destroyed with the completion of the Clairborne and Millers Ferry lock and dams in the early 1970s (Shepard et. al., 1997; Bennett et. al., 2008). Specimens have not been collected from this area since.

The Conasauga population has declined precipitously in the past two decades, has not been observed since 2000 (Freeman et al. 2017), and may be extirpated.

The Etowah population is still extant in the mainstem above Allatoona Reservoir and the lower reaches of Amicalola Creek. It can be locally abundant, across roughly 40 river miles of river from Canton upstream to Silver City, although some evidence suggests a decline over this range (Freeman et al. 2017). The upstream limit appears to be constrained by the edge of the Blue Ridge escarpment.

Surveys during 2018 at 13 sites (7 in the Cahaba, 6 in the Tombigbee drainage) resulted in observations of *N. munitus* at 4 sites in the Cahaba (with 34 fin clips taken for future genetic analysis) and 3 sites in the Tombigbee (with 22 fin clips taken for genetic analysis) Collection data for these sites are given in Appendix 1.

Fig. 1. Truss and other measurements used in morphometric analysis. See Methods for description of each measurement.

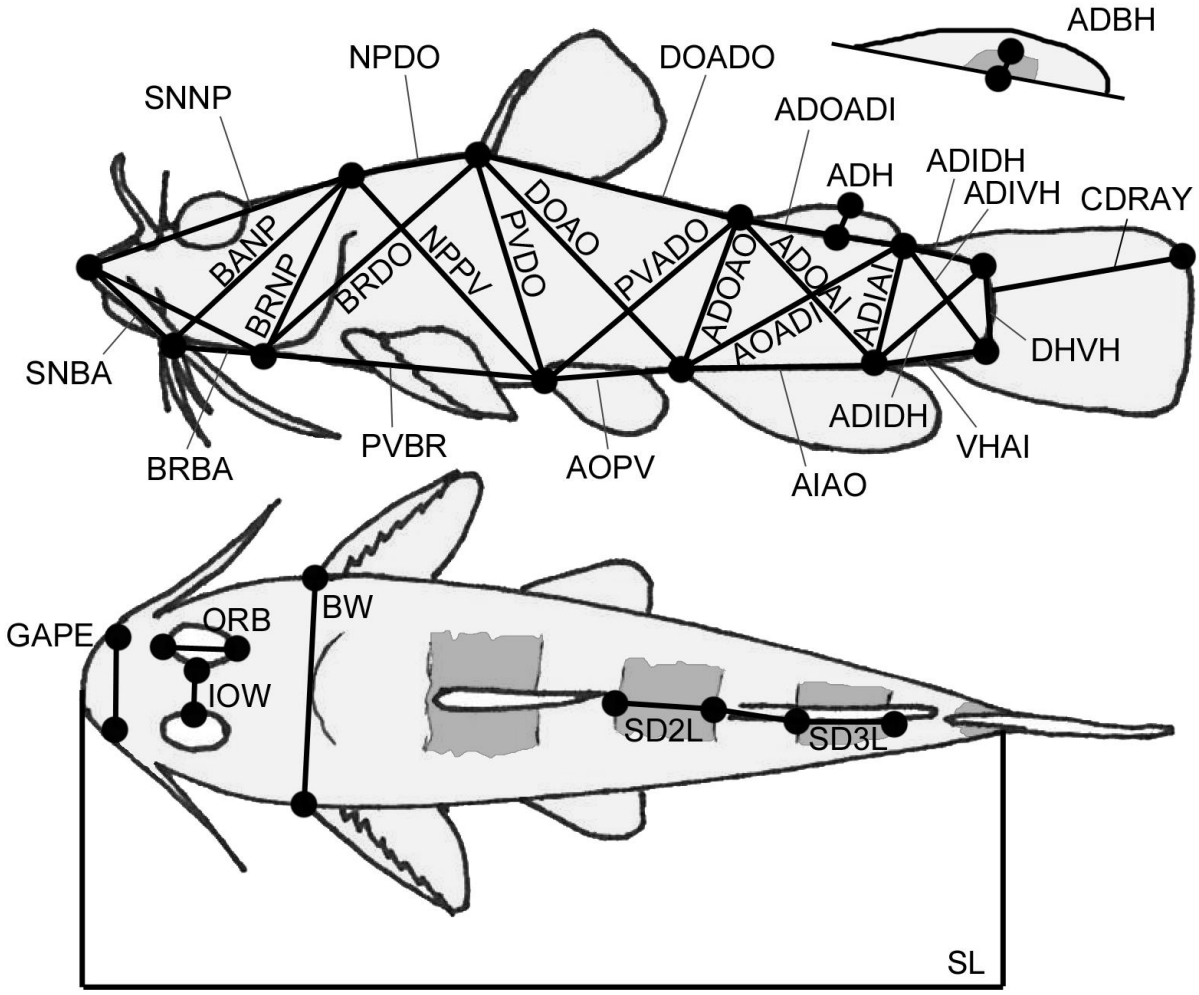

Fig. 2. Sheared principal component analysis of mensural characters, males only, with adipose bar height (ADBHT) and saddle characters (SD2L and SD3L) included. Variables loading strongest on sPC2 are ADBH (0.714), SD2L (0.363), and SD3L (-0.377).

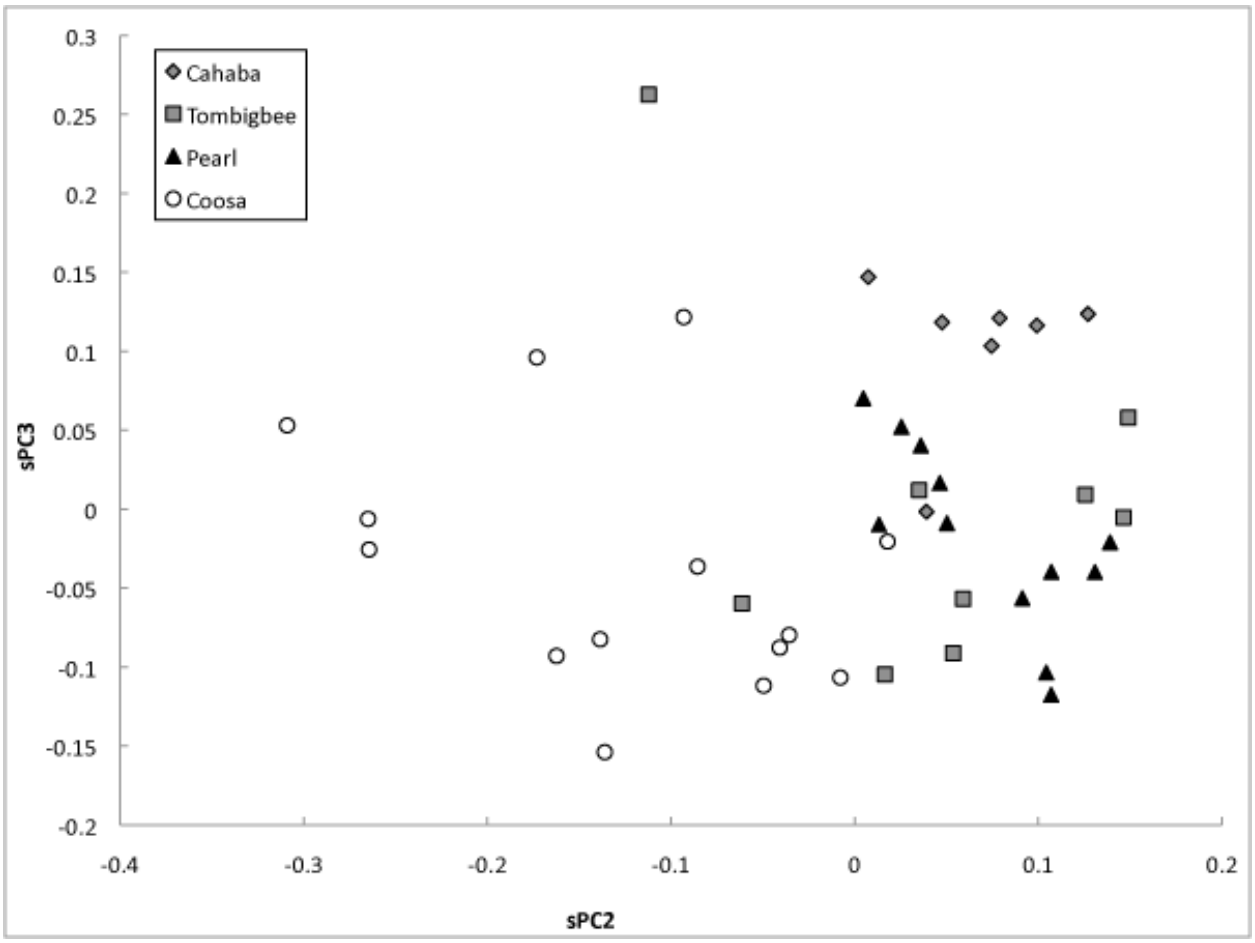

Fig. 3. Sheared principal component analysis of mensural characters, females only, with adipose bar height (ADBHT) and saddle characters (SD2L and SD3L) included. Variables loading strongest on sPC2 are ADBH (0.481), SD2L (-0.428), and SD3L (0.422).

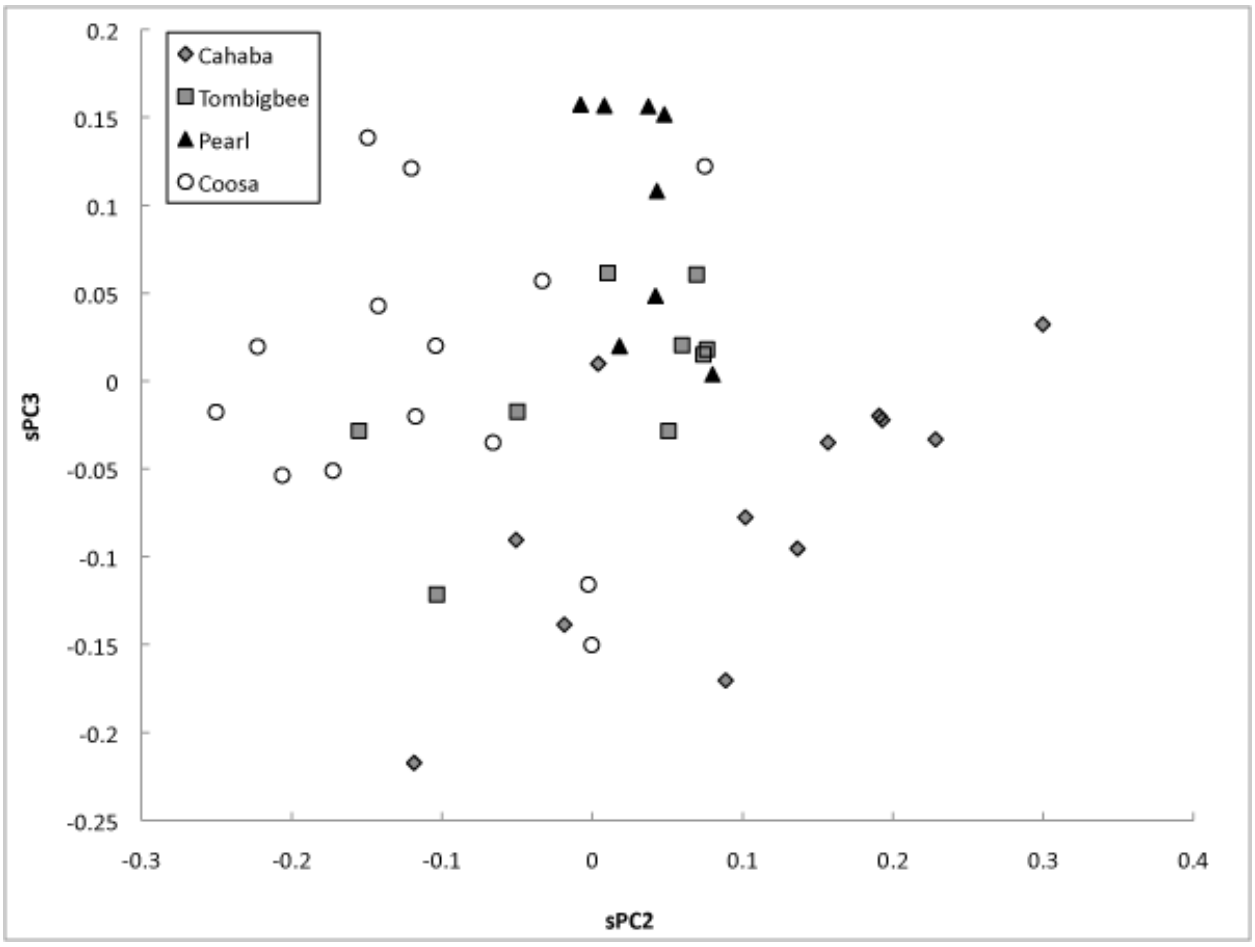

Fig. 4. Sheared principal component analysis of mensural characters, males only, with saddle characters and adipose bar height excluded. Variables loading strongest on sPC2 are ADIDH (0.493) and IOW (-0.331). Variables loading strongest on sPC3 are SNBA (0.448), AIAO (-0.357), and GAPE (0.354).

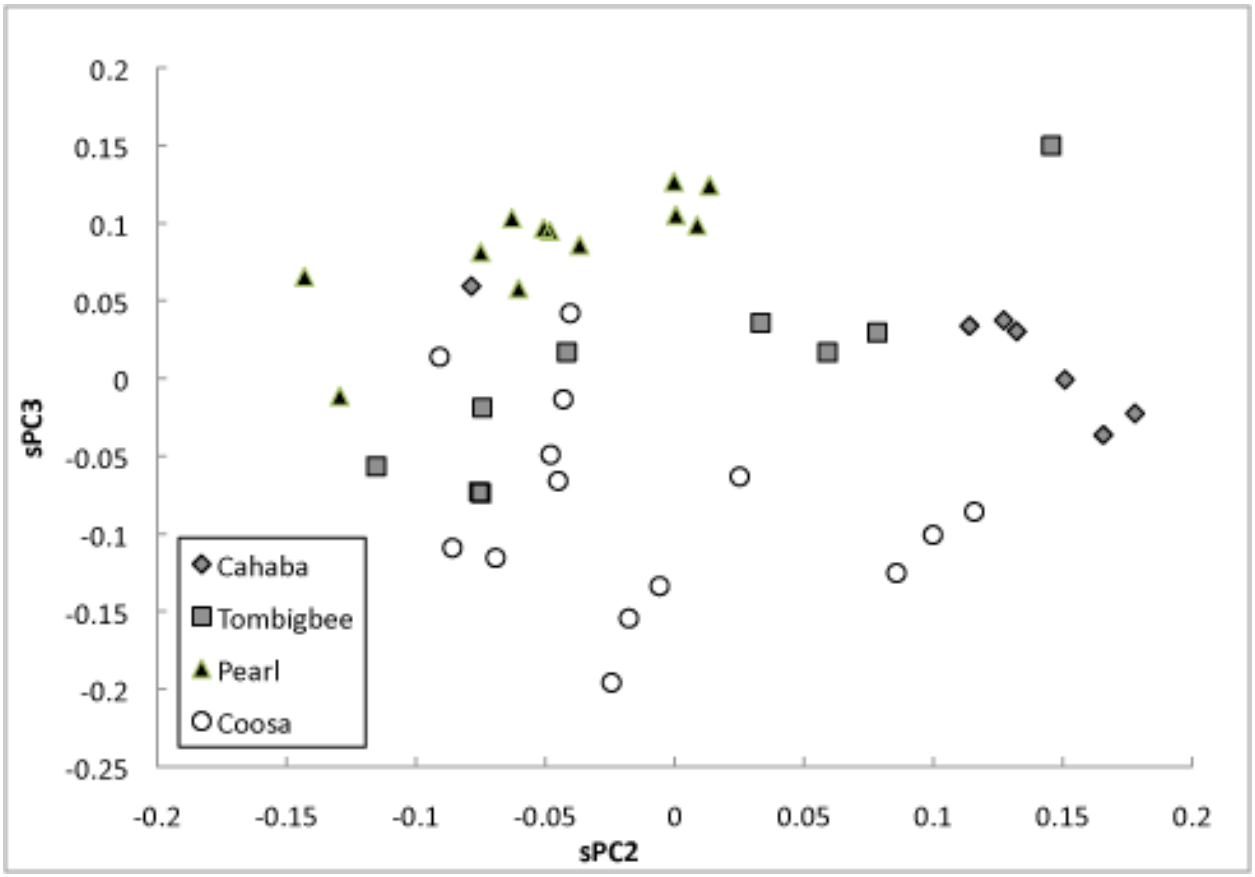

Fig. 5. Sheared principal component analysis of mensural characters, females only, with saddle characters and adipose bar height excluded. Variables loading strongest on sPC2 are NPDO (-0.477), ADIDH (-0.364), and BABR (0.353). Variables loading strongest on sPC3 are SNBA (0.387) and AOPV (-0.319).

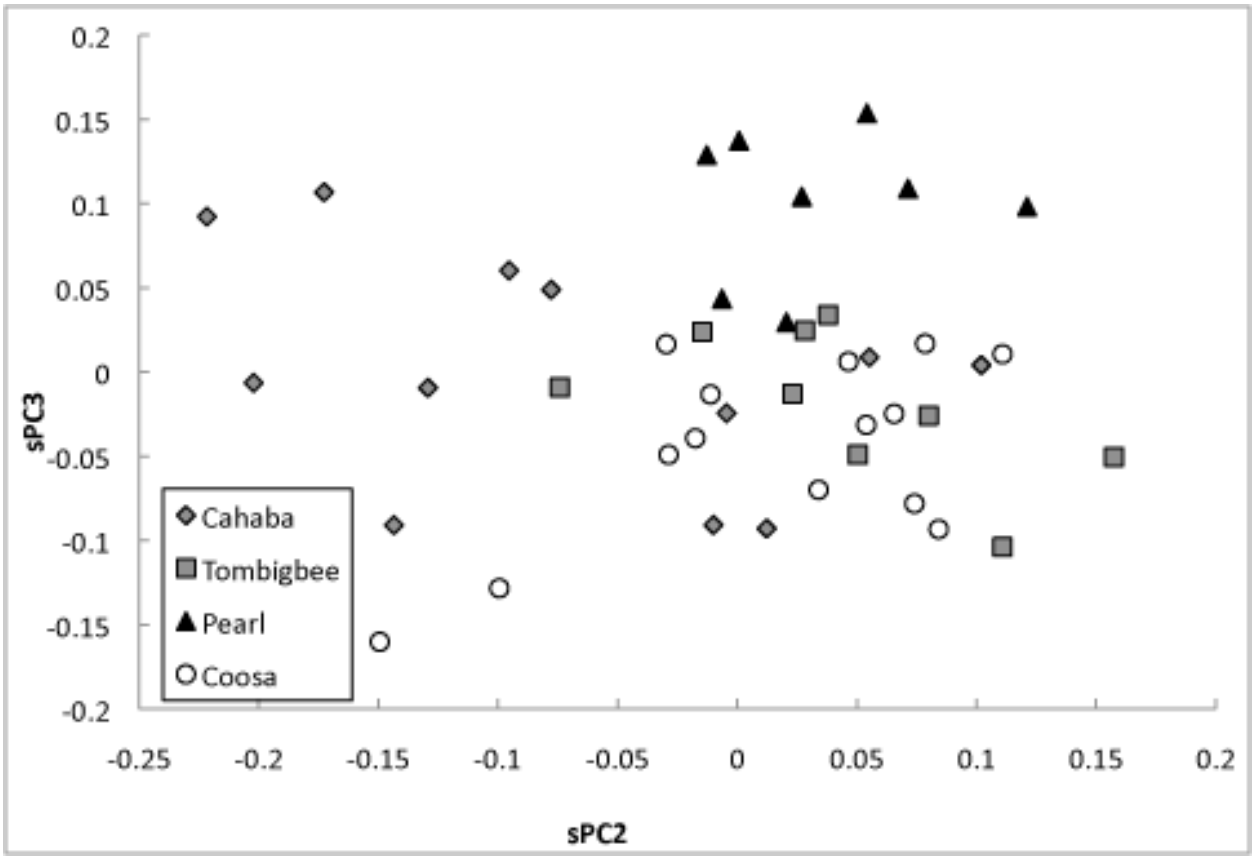

Fig. 6. One of six equally parsimonious trees resulting from heuristic MP search.

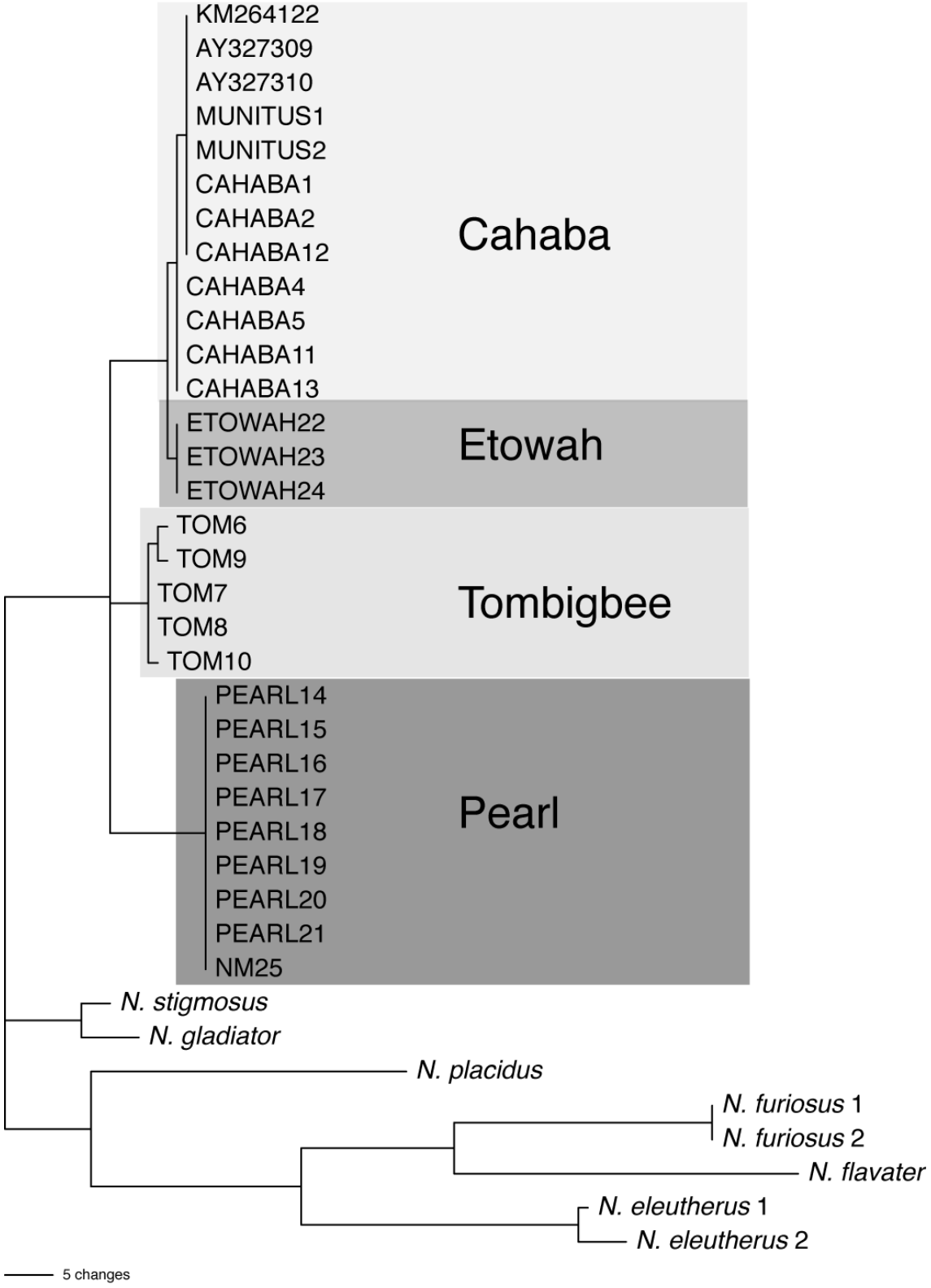

Fig. 7. Bootstrap and jackknife consensus trees were identical; bootstrap support is given above each node, jackknife support below each node. Drainage shading follows that given in Fig. 6.

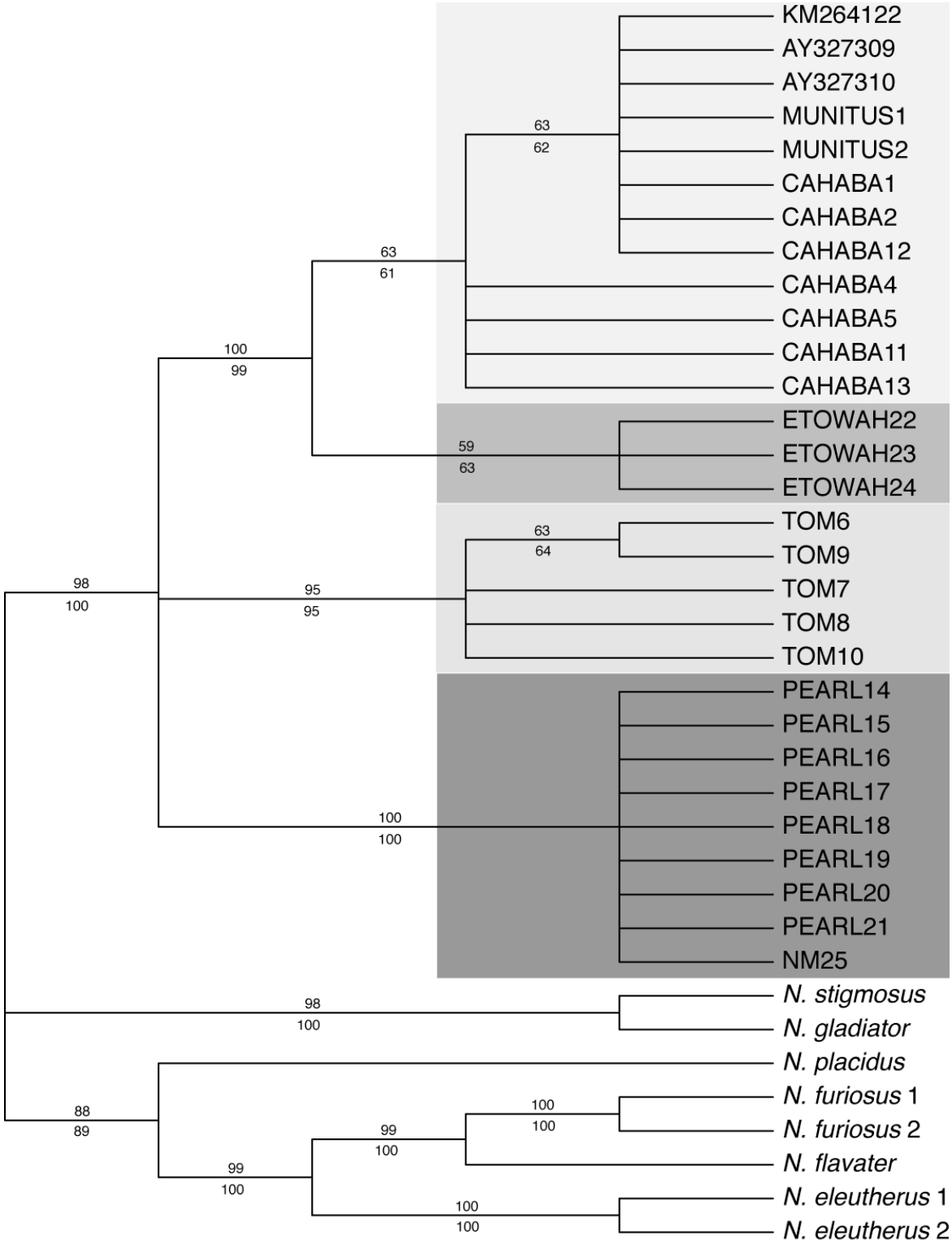

Fig. 8. *Noturus munitus*, Cahaba River, Perry Co., AL.

Fig. 9. Cahaba River, Perry Co., AL; showing extensive gravel bar habitat utilized by *Noturus munitus*.

Fig. 10. *Noturus munitus*, Buttahatchee River, Lamar Co., AL.

Fig. 11. Sipsey River, Pickens Co., AL; habitat in this system is patchy, but extensive.

Fig. 12. Buttahatchee River, Lamar Co., AL. Frecklebelly madtoms at this site were associated with patches of coarse woody debris over coarse gravel.

Fig. 13. Length-frequency analysis of 54 specimens of *Noturus munitus* from the Etowah River.

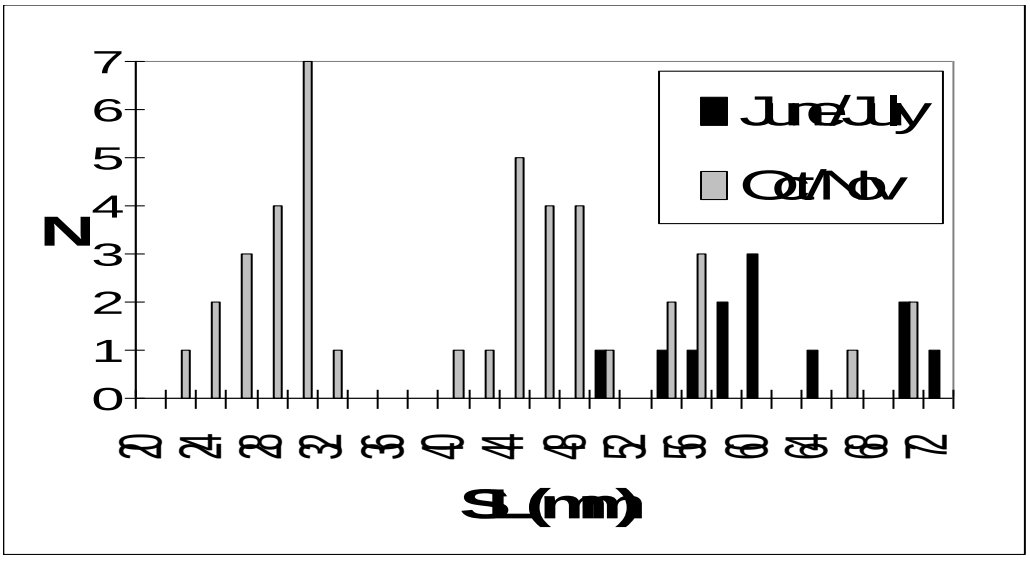

Fig. 14. Length-frequency analysis of 206 specimens of *Noturus munitus* from the Tombigbee River system.

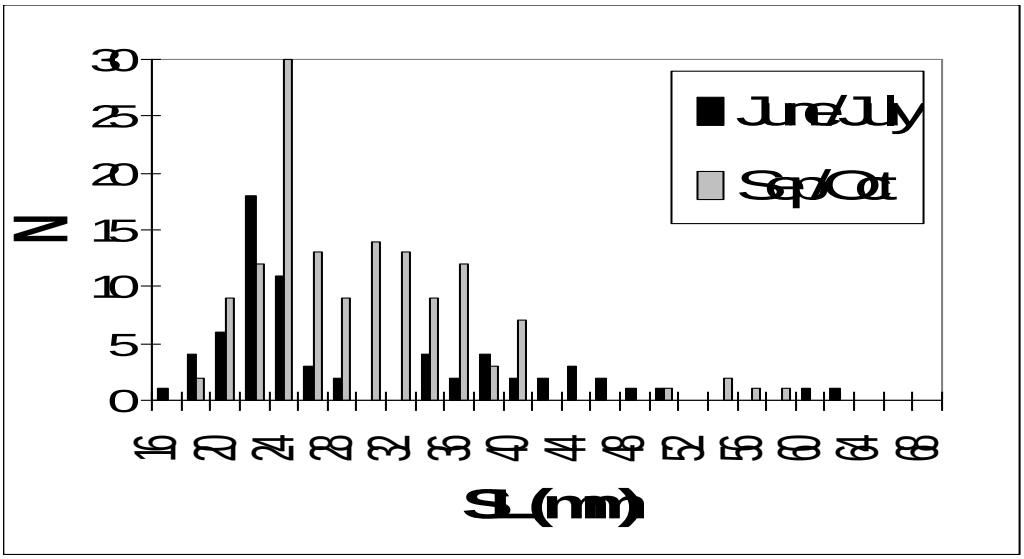

Table 1. Variance loadings for principal components of morphometric variables for male *Noturus munitus,* with adipose bar height and saddle length variables included.

		Sheared	
Variable	Size	PC2	PC3
SL	-0.158	-0.038	-0.005
SNNP	-0.169	0.015	-0.086
NPDO	-0.187	0.151	0.378
DOADO	-0.195	-0.075	-0.154
ADOADI	-0.141	0.024	0.021
ADIDH	-0.190	-0.135	0.506
DHVH	-0.173	-0.140	-0.173
VHAI	-0.144	-0.035	0.131
AIAO	-0.142	-0.135	-0.091
AOPV	-0.185	-0.059	0.042
PVBR	-0.155	-0.047	-0.018
BRSN	-0.203	0.065	0.112
SNBA	-0.184	0.082	-0.010
BANP	-0.156	0.054	-0.218
BABR	-0.196	-0.103	0.114
BRDO	-0.163	0.011	-0.035
NPPV	-0.160	-0.020	0.086
PVADO	-0.172	-0.064	-0.043
DOAO	-0.159	-0.046	-0.030
ADOAI	-0.149	0.034	0.083
AOADI	-0.143	-0.103	-0.002
ADIVH	-0.177	-0.061	0.067
AIDH	-0.160	-0.094	0.045
ADH	-0.142	0.092	-0.066
ADBH	-0.206	0.714	-0.232
SD2L	-0.211	-0.377	-0.280
SD3L	-0.167	0.363	0.061
BRNP	-0.170	-0.079	-0.150
PVDO	-0.154	-0.013	-0.032
ADOAO	-0.166	-0.050	0.051
ADIAO	-0.149	-0.124	0.067
CDRAY	-0.141	-0.126	-0.232
ORB	-0.160	0.101	-0.157
IOW	-0.172	0.035	-0.179
GAPE	-0.177	0.077	0.366

Table 2. Variance loadings for principal components of morphometric variables for female *Noturus munitus,* with adipose bar height and saddle length variables included.

		Sheared	
Variable	Size	PC2	PC3
SL	-0.169	-0.044	-0.016
SNNP	-0.157	-0.101	0.067
NPDO	-0.200	0.262	-0.142
DOADO	-0.191	-0.132	0.109
ADOADI	-0.173	0.047	-0.080
ADIDH	-0.202	0.091	-0.256
DHVH	-0.146	-0.246	-0.185
VHAI	-0.176	0.104	-0.032
AIAO	-0.148	-0.098	-0.001
AOPV	-0.154	-0.029	-0.094
PVBR	-0.173	-0.065	0.030
BRSN	-0.176	-0.068	0.034
SNBA	-0.176	0.033	0.112
BANP	-0.151	-0.107	0.084
BABR	-0.191	-0.233	0.228
BRDO	-0.156	-0.027	0.026
NPPV	-0.184	0.047	-0.016
PVADO	-0.162	-0.028	0.052
DOAO	-0.169	0.014	-0.049
ADOAI	-0.154	0.035	-0.206
AOADI	-0.151	-0.023	-0.053
ADIVH	-0.159	-0.059	-0.029
AIDH	-0.143	-0.018	-0.077
ADHT	-0.167	0.243	0.398
ADBHT	-0.178	0.481	0.576
SD2L	-0.213	-0.428	0.122
SD3L	-0.192	0.422	-0.365
BRNP	-0.151	-0.141	0.085
PVDO	-0.168	-0.002	-0.045
ADOAO	-0.158	-0.002	-0.083
ADIAO	-0.131	0.018	-0.112
CDRAY	-0.160	-0.134	0.061
ORB	-0.134	-0.101	0.088
IOW	-0.161	0.004	-0.050
GAPE	-0.201	0.144	-0.182

Table 3. Proportional ratios of adipose fin bar and saddle length characters.

Adipose fin bar/adipose fin height ratio.

	Cahaba	Tombigbee	Pearl	Coosa
mean	0.934	0.954	0.100	0.804
range	0.652-1.0	0.588-1.0	1.0	0.555-1.0
sd	0.0114	0.100	0	0.157

Saddle II length / SL.

	Cahaba	Tombigbee	Pearl	Coosa
mean	0.094	0.094	0.097	0.120
range	0.073-0.104	00.071-0.111	0.084-0.114	0.086-0.152
sd	0.008	0.013	0.010	0.017

Saddle III length/ SL.

	Cahaba	Tombigbee	Pearl	Coosa
mean	0.081	0.072	0.070	0.063
range	0.051-0.108	0.054-0.094	0.056-0.086	0.048-0.096
sd	0.012	0.012	0.009	0.010

Table 4. Variance loadings for principal components of morphometric variables for male *Noturus munitus,* with adipose bar height and saddle length variables excluded.

		Sheared	
Variable	Size	PC2	PC3
SL	-0.16566	-0.02220	-0.00970
SNNP	-0.17649	-0.13557	0.05610
NPDO	-0.19363	0.39005	0.21555
DOADO	-0.20384	-0.08339	-0.15848
ADOADI	-0.14636	0.03253	-0.06049
ADIDH	-0.20010	0.49341	0.16225
DHVH	-0.18204	-0.11982	-0.16493
VHAI	-0.14859	0.13826	0.02862
AIAO	-0.14821	0.01092	-0.35674
AOPV	-0.19363	0.02847	-0.11672
PVBR	-0.16318	-0.04081	-0.00645
BRSN	-0.19118	-0.08718	0.09966
SNBA	-0.19951	-0.16802	0.44781
BANP	-0.16278	-0.24204	0.04466
BABR	-0.19723	-0.11477	0.26568
BRDO	-0.16985	-0.06244	0.04040
NPPV	-0.16605	0.09346	0.03076
PVADO	-0.18318	-0.00195	-0.16226
DOAO	-0.16846	0.01018	-0.13190
ADOAI	-0.15336	0.17283	-0.10713
AOADI	-0.15327	0.05122	-0.25477
ADIVH	-0.19000	0.08043	-0.08732
AIDH	-0.17308	0.03532	-0.07738
ADHT	-0.14598	0.02440	-0.13126
BRNP	-0.17891	-0.24259	0.05871
PVDO	-0.16071	0.01714	-0.04899
ADOAO	-0.17250	0.13062	-0.18311
ADIAO	-0.15527	0.14208	-0.21126
CDRAY	-0.14725	-0.19400	-0.19174
ORB	-0.16728	-0.25447	0.14893
IOW	-0.18223	-0.33060	0.09984
GAPE	-0.18566	0.26444	0.35370
BW	-0.18992	-0.04552	0.09845

Table 5. Variance loadings for principal components of morphometric variables for female *Noturus munitus,* with adipose bar height and saddle length variables excluded.

		Sheared	
Variable	Size	PC2	PC3
SL	-0.17534	0.05258	-0.03636
SNNP	-0.16476	0.17276	0.05675
NPDO	-0.18896	-0.47694	0.07031
DOADO	-0.19676	0.13996	0.08347
ADOADI	-0.17996	-0.01428	-0.13785
ADIDH	-0.21193	-0.36382	0.00629
DHVH	-0.17349	0.20660	-0.18502
VHAI	-0.18331	-0.18342	0.05009
AIAO	-0.15252	0.07153	-0.17981
AOPV	-0.17288	0.03037	-0.31924
PVBR	-0.18509	0.12622	-0.06443
BRSN	-0.18355	0.09747	0.15521
SNBA	-0.18430	0.02228	0.38742
BANP	-0.15717	0.18146	0.07631
BABR	-0.19346	0.35335	0.27217
BRDO	-0.16209	0.02652	-0.02588
NPPV	-0.18994	-0.09092	0.02156
PVADO	-0.17462	-0.02101	-0.01361
DOAO	-0.17569	-0.06161	-0.07855
ADOAI	-0.15881	-0.10047	-0.25767
AOADI	-0.15629	-0.03266	-0.09200
ADIVH	-0.17020	0.01220	-0.06410
AIDH	-0.14917	-0.02072	-0.14280
ADHT	-0.17018	-0.12126	0.59459
BRNP	-0.16663	0.23062	0.00583
PVDO	-0.17398	-0.08497	-0.11655
ADOAO	-0.16479	-0.11230	-0.11872
ADIAO	-0.13689	-0.13710	-0.13782
CDRAY	-0.16607	0.20377	-0.02645
ORB	-0.14027	0.26388	0.00490
IOW	-0.16593	0.04076	-0.06953
GAPE	-0.21108	-0.27458	0.13590
BW	-0.18080	0.04477	-0.05197

Table 6. Proportional ratios of variables with consistent heavy loadings in the sPCA for both sexes, adipose bar and saddle varibles excluded. Both SNBA and ADIDH are divided into SL.

	SNBA			
	Cahaba	Tombigbee	Pearl	Coosa
mean	0.083	0.077	0.090	0.077
range	0.068-0.102	0.057-0.093	0.079-0.102	0.063-0.093
sd	0.009	0.009	0.006	0.006

	ADIDH			
	Cahaba	Tombigbee	Pearl	Coosa
mean	0.087	0.086	0.086	0.091
range	0.065-0.111	0.065-0.109	0.060-0.102	0.073-0.115
sd	0.011	0.012	0.013	0.010

Table 7. Meristic variation in *Noturus munitus*.

Dorsal fin rays	5	6	7	x	SE
Coosa		13	9	6.4	0.11
Cahaba	3	15	2	6.0	0.12
Tombigbee	1	16	2	5.8	0.08
Pearl		14	6	6.3	0.11

Pectoral fin rays	6	7	8	9	x	SE
Coosa		11	10	1	7.5	0.13
Cahaba	1	9	9		7.4	0.14
Tombigbee	3	12	2	1	6.7	0.17
Pearl	1	16	3		7.1	0.10

Pelvic fin rays	7	8	9	10	x	SE
Coosa	1	13	8		8.3	0.12
Cahaba	1	7	8	3	8.7	0.18
Tombigbee	2	11	5		7.7	0.15
Pearl	5	10	4		8.0	0.15

Anal fin rays	11	12	13	14	15	x	SE
Coosa		3	11	7	1	13.2	0.16
Cahaba		4	8	4	2	13.3	0.23
Tombigbee	1	7	6	4		12.1	0.21
Pearl	2	6	5	6	1	12.9	0.25

Posterior pectoral serrae

	7	8	9	10	11	x	SE
Coosa	1	7		2		8.3	0.25
Cahaba	1	7	7	3		8.7	0.23
Tombigbee		7	7	2	1	8.8	0.21
Pearl	6	6	8			8.1	0.20

Table 7. Meristic variation in *Noturus munitus,* cont'd.

Anterior pectoral serrae

	11	12	13	15	16	17	18	19	20	21	22	23	24	25	x
Coosa				1	1		3	2	2	1					18
Cahaba	1		1			3	2		2	5	1	1	1	1	19
Tombigbee					1			2	1	2	5	4	2	1	21
Pearl		1				1	1	2	4	3	3		4	1	21

Lateral line pores

	21	23	25	26	27	28	29	30	31	32	33	34	35	x	SE
Coosa	1	1	3		5	2	3	2	3	1				28.0	0.62
Cahaba					1	2	4	3	3	1	3			29.5	0.63
Tombigbee			1		2	3		3	2	2	1	1	1	28.3	0.71
Pearl			1	1	5	2	2	3	1	2	1			29.1	0.53

Table 8. Pairwise uncorrected p-distances within (diagonal) and between (below diagonal) sampled populations.

	Etowah	Cahaba	Tombigbee	Pearl
Etowah	0			
Cahaba	0.002-0.003	0.0-0.001		
Tombigbee	0.009-0.011	0.009-0.012	0-0.003	
Pearl	0.015-0.016	0.015-0.016	0.011-0.014	0

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Appendix 1. Field data for 2018 collections. [7 in Cahaba, 6 in Tombigbee]

DAN18-91 AL: Bibb County, Cahaba River, bar on river right at end of Goodson Road (several gates and through fields to access). 32.88795 -87.16786. 23 Aug 2018. 1100-1130. DA Neely, SM Mitchell. BPE, 10x4 ft seine. No *N. munitus* observed; mediocre habitat, silty gravel. ALL released: *Campostoma oligolepis, Cyprinella venusta, Notropis stilbius, Notropis uranoscopus, Notropis volucellus, Pimephales vigilax, Hypentelium etowanum, Moxostoma carinatum, Moxostoma duquesnei, Gambusia affinis, Lepomis megalotis, Micropterus henshalli*. [no darters! not even *P. nigrofasciata*!]

DAN18-92 AL: Bibb County, Cahaba River, gravel bar on river right ca. 0.75 mi upstream of Harrisburg Bridge. 32.85875 -87.18978. 23 Aug 2018. 1300-1345. DA Neely, SM Mitchell. BPE, 10x4 ft seine. No *N. munitus* observed; Nice habitat, not convinced they're not there. ALL released: *Campostoma oligolepis, Cyprinella venusta, Hybopsis winchelli, Notropis atherinoides, Notropis volucellus, Pimephales vigilax, Ictalurus punctatus, Gambusia affinis, Lepomis megalotis, Micropterus henshalli, Ammocrypta beani, Etheostoma chlorosomum, Etheostoma rupestre, Etheostoma stigmaeum, Etheostoma swaini, Percina nigrofasciata*.

DAN18-93 AL: Bibb County, Cahaba River, gravel bar on river left ca. 0.5 mi upstream of Harrisburg Bridge. 32.85539 -87.19344. 23 Aug 2018. 1400-14:30. DA Neely, SM Mitchell. BPE, 10x4 ft seine. Not great habitat, somewhat silty. All released: *Cyprinella callistia, Cyprinella venusta, Hybopsis winchelli, Notropis stilbius, Notropis uranoscopus, Notropis volucellus, Lepomis megalotis, Micropterus henshalli, Ammocrypta beani, Ammocrypta meridiana, Etheostoma rupestre, Etheostoma stigmaeum, Percina nigrofasciata*.

DAN18-94 AL: Bibb County, Cahaba River, gravel bar ca. 0.5 mi downstream of Harrisburg Bridge (roughly = C22). 32.85166 -87.20371. 23 Aug 2018. 1500-1600. DA Neely, SM Mitchell. 10x4 ft seine. *Noturus munitus*, n=15; fin clips from all, one specimen preserved. Released: *Campostoma oligolepis, Cyprinella venusta, Hybopsis winchelli, Notropis ammophilus, Notropis stilbius, Notropis uranoscopus, Ammocrypta beani, Ammocrypta meridiana, Etheostoma rupestre, Percina vigil*.

DAN18-95 AL: Perry County, Cahaba River, gravel bar ca. 0.2 mi downstream from CR14 at Sprott (roughly = C10). 32.66545 -87.24107. 23 Aug 2018. 1930-2030. DA Neely, SM Mitchell. 10x4 ft seine. *Noturus munitus*, n=8; fin clips from all. All released: *Cyprinella venusta, Notropis ammophilus, Notropis uranoscopus, Notropis volucellus, Phenacobius catostomus, Pimephales vigilax, Carpioides velifer (YOY), Ictalurus punctatus (YOY), Micropterus henshalli, Ammocrypta beani, Ammocrypta meridiana, Etheostoma rupestre, Percina nigrofasciata, Percina vigil*.

DAN18-96 AL: Perry County, Cahaba River, gravel bar ca. 0.2 mi downstream of CR6 at Suttle (roughly = C4). 32.52831 -87.19787. 24 Aug 2018. 0745-0845. DA Neely, SM Mitchell. 10x4 ft seine. *Noturus munitus*, n=5; fin clips from all. All released: *Campostoma oligolepis, Cyprinella venusta, Hybopsis winchelli, Notropis ammophilus, Notropis atherinoides, Notropis uranoscopus, Notropis volucellus, Pimephales vigilax, Carpioides velifer YOY, Moxostoma carinatum* (several large adults observed but not captured), *Ictalurus*

punctatus (YOY), Gambusia affinis, Lepomis megalotis, Micropterus henshalli, Ammocrypta beani, Ammocrypta meridiana,Etheostoma histrio, Etheostoma rupestre, Percina nigrofasciata.

DAN18-97 AL: Dallas County, Cahaba River, gravel bar on river right ca. 0.75 mi downstream from US Hwy 80. 32.43575 -87.19028. 24 Aug 2018. 1100-1200. DA Neely, SM Mitchell. 10x4 ft seine. *Noturus munitus*, n=6; fin clips from all. ALL released: *Campostoma oligolepis, Cyprinella venusta, Hybopsis winchelli, Notropis atherinoides, Notropis volucellus, Pimephales vigilax, Carpioides YOY, Ictalurus punctatus (YOY), Gambusia affinis, Lepomis megalotis, Micropterus henshalli, Ammocrypta beani, Etheostoma chlorosomum, Etheostoma rupestre, Etheostoma stigmaeum, Etheostoma swaini, Percina nigrofasciata*.

DAN18-98 AL: Tuscaloosa County, Sipsey River along old US Hwy 82 (now CR 140) between Buhl and Elrod; shoal area near 33.25651 -87.77374. 29 Aug 2018. 1145-1300. DA Neely, BR Kuhajda, SM Mitchell, MW Pugh. Backpack shocker, 10x6ft seine. Water slightly turbid; width to 20m, depth to >2m; current slow; substrate sand, CWD, detritus. No vegetation. Most released: *Cyprinella venusta, Lythrurus bellus, [Hybopsis amnis*, 1 retained, .04], *Notropis texanus, Pimephales vigilax, Moxostoma poecilurum, Ictalurus punctatus, Noturus leptacanthus, Pylodictis olivaris, Gambusia affinis, Labidesthes vanhyningi, Lepomis macrochirus, Lepomis megalotis, Ammocrypta beani, Etheostoma chlorosoma, Etheostoma histrio* [1 retained, .01], *Etheostoma lachneri, Etheostoma rupestre, Etheostoma swaini, Percina lenticula* [1 retained, .02], *Percina nigrofasciata, Percina sciera, Percina vigil* [1 retained, .03].

DAN18-99 AL: Lamar County, Buttahatchee River at AL Hwy 17 NW of Sulligent. 33.91899 -88.14642. 29 Aug 2018. 1800-1840. DA Neely, BR Kuhajda, SM Mitchell. Backpack shocker, 10x6ft seine. Water slightly turbid; width to 25m, depth to >2m; current slow-moderate; substrate sand, gravel, CWD, detritus. No vegetation. Most released: *Cyprinella venusta, Cyprinella ?, Noturus munitus* [2; large= .01, small= .02], *Etheostoma lachneri, Percina sciera, Percina vigil*.

DAN18-100 AL: Lamar County, Buttahatchee River at gravel bar upstream of CR 16, E of Henson Springs. 34.02075 -88.05345. 29 Aug 2018. 1940-2015. DA Neely, BR Kuhajda, SM Mitchell. Backpack shocker, 10x6ft seine. Water slightly turbid; width to 20m, depth to >1m; current slow-moderate; substrate sand, gravel, CWD, detritus. No vegetation. Most released: *Campostoma oligolepis, Cyprinella callistia, Cyprinella venusta, Notropis ammophilus, Notropis texanus, Notropis ?, Pimephales vigilax, Hypentelium etowanum, Ictalurus punctatus, Noturus leptacanthus* [2 in formalin], *Noturus munitus* [12, fin clips from all], *Etheostoma lachneri, Etheostoma nigrum, Etheostoma rupestre, Etheostoma swaini*, *Percina nigrofasciata, Percina sciera, Percina vigil*.

DAN18-101 AL: Pickens County, Sipsey River at first gravel bar downstream of AL Hwy 14. 33.04011 -88.11375. 30 Aug 2018. 1130-1210. DA Neely, BR Kuhajda, SM Mitchell, P Nensteil. Backpack shocker, 10x6ft seine. Backpack shocker, 10x6ft seine. Water slightly turbid; width to 20m, depth to >2m; current slow; substrate sand, gravel, CWD, detritus. Sparse *Justicia* along margins. Most released: *Cyprinella venusta, Notropis texanus,*

Hypentelium etowanum, Strongylura marinus (observed only), *Ammocrypta beani, Crystallaria asprella* [1 retained], *Percina kathae, Percina nigrofasciata*.

DAN18-102 AL: Pickens County, Sipsey River at second gravel bar complex downstream of AL Hwy 14. 33.04128 -88.12203. 30 Aug 2018. 1245-1330. DA Neely, BR Kuhajda, SM Mitchell, P Nensteil. Backpack shocker, 10x6ft seine. Backpack shocker, 10x6ft seine. Water slightly turbid; width to 20m, depth to >2m; current slow; substrate sand, gravel, CWD, detritus. Sparse *Justicia* along margins. Most released: *Cyprinella venusta, Notropis stilbius, Labidesthes vanhyningi, Lepomis macrochirus, Lepomis megalotis, Micropterus henshalli, Crystallaria asprella* (abundant! 25+, 4 in one short seine haul!), *Percina kathae, Percina vigil*.

DAN18-103 AL: Pickens County, Sipsey River at 1st major gravel bar upstream of AL Hwy 14. 33.03422 -88.10537. 30 Aug 2018. 1430-1530. DA Neely, BR Kuhajda, SM Mitchell, P Nensteil. Backpack shocker, 10x6ft seine. Backpack shocker, 10x6ft seine. Water slightly turbid; width to 25m, depth to >1m; current slow-moderate; substrate sand, gravel, CWD, detritus. Sparse *Justicia* along margins. Most released: *Cyprinella venusta, Macrhybopsis boschungi* [2 retained], *Hypentelium etowanum, Noturus leptacanthus, Noturus munitus* [fin clips from 8, all released], *Gambusia affinis, Lepomis megalotis, Lepomis macrochirus, Percina nigrofasciata, Percina sciera,*