# Two New Species of *Noturus* (Siluriformes: Ictaluridae) from the Tennessee River Drainage: Description, Distribution, and Conservation Status

BROOKS M. BURR, DAVID J. EISENHOUR, AND JAMES M. GRADY

Nominotypical *Noturus elegans* are herein restricted to the Green River drainage of central Kentucky and north-central Tennessee. Ten specimens and additional new material from the Duck River originally allocated to *N. elegans* are here described as a new species, *Noturus fasciatus*, restricted to the Duck River system and two minor tributaries of the lower arm of the Tennessee River, Tennessee. A second new species, *Noturus crypticus*, is described from the only known extant population in Little Chucky Creek, Greene County, Tennessee. These three taxa are closely related and are distinguished on the basis of modal differences in anal-fin ray numbers, body shape, pigmentation, and genotypic arrays. *Noturus crypticus* is described from only eight specimens, is clearly uncommon, probably relict, and in need of federal protection and future propagation efforts. All three species are primarily riffle/glide dwellers in smallto medium-sized streams where they may be found in or under gravel, rubble, and slab rock. The limited geographic distributions of the two new species places them at greater risk of extinction, warranting comprehensive evaluation of their life histories, demographic characteristics, recruitment rates, and nesting requirements.

**ORPHOLOGICAL** conservatism confounds taxonomic research, particularly efforts to establish and test species limits. Among North American freshwater fishes, morphological conservatism is particularly problematic for madtom (Noturus) catfish taxonomy. Nearly one half of the known taxonomic diversity of madtom catfishes was described in the single comprehensive revision of the group, in which Taylor (1969) established that variation for standard meristic and morphometric traits is limited within and among species. The more conspicuously variable attributes of these secretive fishes are features of pigmentation, which also are more difficult to quantify and often vary according to substrate and water quality (Taylor, 1969). Despite repeated suggestion of additional taxonomic diversity (at least ten formally undescribed forms) among madtoms (Etnier and Jenkins, 1980; Grady and LeGrande, 1992; Burr and Stoeckel, 1999), only three additional species, N. stanauli (Etnier and Jenkins, 1980), N. taylori (Douglas, 1972), and N. gladiator (Thomas and Burr, 2004) have been described since Taylor's (1969) revision. These species have small to tiny or fragmented ranges, and aspects of pigmentation are important diagnostic characters of each. Genetic (Grady and LeGrande, 1992; Hardman, 2004; Grady, pers. obs.) and karyotypic data (LeGrande, 1981; Grady and LeGrande, 1992) confirmed the hypothesis of morphological conservatism among madtoms by outlining population structure suggestive of speciation within taxa for which Taylor (1969) found little morphological variation.

Populations assigned to the elegant madtom, a member of the subgenus Rabida (the "saddled" or "mottled" madtoms, sensu Taylor [1969]), are among the putative sources of additional taxonomic diversity. The type locality of Noturus elegans is in the Green River drainage, Kentucky, but Taylor (1969) presented descriptive data on ten individuals from the Duck River system, Tennessee, that he also allocated to N. elegans. These individuals were more boldly pigmented and had modally higher anal-fin ray counts than nominotypical N. elegans. Collection (Eisenhour et al., 1996) and analysis of additional material from the Duck River system and adjacent tributaries of the lower Tennessee River indicated that what Taylor had so few specimens of is, in reality, an undescribed species of Noturus that was previously (Warren et al., 2000) referred to as the Saddled Madtom. In addition to trenchant phenotypic traits, the Saddled Madtom differs substantially from N. elegans in fixed allozymic electromorphs. Recent collection and analysis of specimens of Noturus elegans from the Tennessee River drainage also suggested additional undescribed taxonomic diversity. Specimens collected by Charles F. Saylor from Little Chucky Creek, Greene County, Tennessee, were provisionally identified as N. elegans but were thought to represent an undescribed species of Noturus (Etnier and Starnes [1993] for additional comments). Six additional specimens of this putative undescribed species, previously (Boschung and Mayden, 2004) referred to as the Chucky Madtom, also from Little Chucky Creek, were collected during three conservation

status surveys (B. M. Burr and D. J. Eisenhour, pers. obs.; P. W. Shute et al., pers. comm.; N. Lang et al., pers. comm.).

Taxonomic interpretations of intra- versus interpopulation variation, notably whether divergence is sufficient to warrant species recognition, are confounded by the number of species concepts and their narrow focus within the evolutionary continuum of divergence (Mayden, 1997; Johnson et al., 2004). Assessing variation in evolutionarily independent characters (Avise and Ball, 1990) addresses the problem of interpreting character variation by considering the effects of divergence across character sets. Concordant partitions in evolutionarily independent characters is strong evidence of lineage independence (Avise and Ball, 1990; Wiens and Penkrot, 2002) and would be predicted under many species concepts. The value of a multifaceted assessment of variation to the discovery and documentation of fish diversity has been demonstrated in descriptions of species such as Notropis albizonatus (Warren et al., 1994), N. cahabae (Mayden and Kuhajda, 1989), and Fundulus bifax (Cashner et al., 1988).

To address the influence of alternative species concepts on interpretations of variation, Johnson et al. (2004) recently suggested that species descriptions should include tests for conformity to the expectations of four general types of species: biological, ecological, phylogenetic, and phenotypic. Applying this rationale, Johnson et al. (2004) presented evidence of concordant partitions in evolutionarily independent data sets, ecological, morphological, and mtDNA characters, across populations of the Leatherside Chub (*Lepidomeda*), which they interpreted as evidence of two species.

Variation in allozymic and morphological characters was assessed within and among populations attributed to Noturus elegans, specifically specimens from the Green River, Duck River, and Little Chucky Creek, to test the hypothesis that each population constitutes a species. Also, mtDNA sequences were used to test for lineage independence and to estimate relationships among populations of N. elegans. Concordant partitions in three evolutionarily independent character sets support recognition of the Saddled and Chucky madtoms as new species, with the name Noturus elegans being restricted to populations in the Green River drainage of central Kentucky and north-central Tennessee. Herein, the geographic ranges, conservation status, and most obvious identifying characters are clarified. Remarks and new descriptive information for additional problematic specimens that have not been convincingly allocated

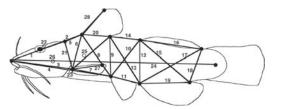


Fig. 1. Truss and standard measurements used in morphometric analyses.

to any known madtom species also are presented.

## MATERIALS AND METHODS

Morphological data.—Counts of fin rays, pores, and vertebrae were made from 182 specimens and follow the methods of Taylor (1969), including the use of soft x-rays (3 A, 15 mv, 30 sec) to allow for accurate counts of anal and caudal rays and vertebrae. Morphometric data analyzed included 28 variables taken from 69 specimens. Measurements were made under a dissecting microscope with calipers to the nearest 0.1 mm and follow the methods of Taylor (1969) and Hubbs and Lagler (1974). Body form was archived with 19 measurements following the truss-geometric protocol (Strauss and Bookstein, 1982; Bookstein et al., 1985) and nine additional measurements (Fig. 1). Multivariate analysis of the morphometric data was accomplished using sheared principal components analysis (PCA) to eliminate overall size effects (Humphries et al., 1981; Bookstein et al., 1985). Principal components were factored from the covariance matrix of log-transformed morphometric characters following the recommendations of Bookstein et al. (1985). Univariate and multivariate analyses were accomplished with Statistical Analysis Systems 6.01 as modified by David L. Swofford, Florida State University.

The intensity of six pigmentation characteristics was estimated for Noturus elegans and the Saddled Madtom to quantify variation in traits that often are diagnostic for madtom species. The features scored were ones that Taylor (1969) used for members of the subgenus Rabida and included: 1) adipose fin pigmentation (absent, at base, intermediate, to edge); 2) anal fin pigmentation (no pigment, at base, pigment in posterior rays, both areas pigmented; 3) saddles on back (do not extend to axial streak [just superficial to horizontal septum] and do not connect, intermediate, extend to axial streak and connect); 4) dark blotch at caudal base (absent or diffuse, diffuse band, well formed crescent); 5) ventral caudal peduncle saddle (absent or diffuse, present with light areas in front and back); and 6) pectoral fin base pigment (no melanophores, small melanophores, large melanophores).

Allozyme data.—Putative N. elegans samples from the Green River (Green R., at Roachville crossing, Green Co., KY; n = 10), Duck River (Brushy Fork Cr., off TN 48, N of Aetna, Hickman Co., TN; n = 10), and Nolichucky River (Little Chucky Creek, Greene Co., TN; n = 4) were scored for variation in 30 presumptive gene loci, encoding 25 enzymatic proteins (Appendix 1). Specimens were placed on dry ice or immersed in liquid nitrogen in the field, transported to the laboratory, and stored at -70 C. Samples of eye, liver, and skeletal muscle tissues were excised from partially thawed specimens and homogenized separately in an equal volume of 0.1 M Tris-HCl, 0.01 M Dithiothreitol (pH 7.0). Homogenates were centrifuged at 5300 g for 20 min at 4 C. Supernatant fractions were screened for variation in allozyme loci using horizontal starch (12% w/v) gel electrophoresis and the Triscitrate (pH 7.5) system of Stein et al. (1985).

MtDNA data.-To assess lineage independence and degree of divergence among populations of *N. elegans*, partial sequences of the mitochondrial Cytochrome b (Cytb) gene were obtained for representatives of each population and used in gene tree reconstructions. Total nucleic acids were extracted from Nolichucky River (Little Chucky Creek, Greene Co., TN; n = 4) specimens with QiAmp tissue extraction columns, following the manufacturer's (Qiagen) protocol for muscle tissue. An  $\sim 850$  bp fragment of Cytb was amplified from genomic DNA extracts, using the universal primers GLUDG-5' and CB3-3' (Palumbi, 1996). Amplifications were conducted in 50  $\mu$ l volumes, which included ~10 ng of total DNA, 10 mM Tris (pH 8.3), 2.5 mM MgCl<sub>2</sub>, 50 mM KCl, 0.01% Triton X-100, 10 pmol of each primer, 200 µM each dNTP, and 2 U of Taq DNA polymerase. PCR conditions were: 4 min at 94 C; 35 cycles of 94 C for 30 s, 48 C for 30 s, 72 C for 30 s, and a final extension of 7 min at 72 C. Amplification products were ethanol precipitated, washed, and resuspended in  $H_2O$ , and 20-50 ng of the purified product was used as template in ABI Big Dye Terminator cycle sequencing reactions. Cycle sequencing reactions were separated on an ABI 3100. Chromatograms were edited and aligned in Sequencher (version 4.1; Gene Codes Corp., Inc.) by eye.

To assess relationships among populations of *N. elegans*, Cytb sequences for Duck, Green, and Nolichucky river populations were combined with Cytb sequences in GenBank for *Noturus* 

and suitable outgroups, notably the data Hardman (2004) used to reconstruct relationships in *Noturus* and sequences from Wilcox et al. (2004). Hardman's (2004) data included complete Cytb sequences for four specimens of putative *N. elegans*, two each from the Green and Duck rivers. Additional specimens from the Duck and Green rivers were characterized for the 850 bp segment of Cytb to confirm Hardman's (2004) findings. The Cytb matrix was trimmed to 804 bp, the length of our partial sequences, to eliminate large blocks of unknown sequence. Parsimony and likelihood Cytb gene trees were reconstructed following the analytical strategies outlined in Hardman (2004).

*Conservation status.*—Conservation status of the Chucky and Saddled madtoms was determined, in part, by surveying selected localities in the French Broad and Duck River systems, respectively, in 1992, 1993, and 1994 using standard minnow seines. To characterize habitat of these species, we measured stream width, depth, and surface velocity at sites of capture. Surface velocity was determined by repeatedly timing a partially submerged object over a given distance. We converted surface velocity to water column velocity by multiplying by 0.8, as described in Orth (1983). Substrate, cover, and land use at each site visited also were recorded.

Synonymies for both new species are skeletal and based only on published articles, not final reports to agencies or other gray literature. Institutional acronyms follow Poss and Collette (1995).

## Noturus fasciatus, new species Saddled Madtom Figure 2A

Noturus elegans Taylor, 1969:150-156 (description, in part; boldness of color pattern and higher anal rays counts noted). Clay, 1975:211 (Duck River system, Tennessee). Etnier and Jenkins, 1980:18, 21 (reference to specimens from Duck and Buffalo rivers appearing different from those in the Barren and Green rivers; rudimentary pelvic ray noted). Rohde, 1980:450 (range, in part; Duck River system, Tennessee). Ramsey et al., 1984:8 (in part, rare in northern Alabama). Burr and Warren, 1986:175 (Tennessee range shown on map). Starnes and Etnier, 1986:343 (in Cumberland and Tennessee River drainages). Page and Burr, 1991:205, Map 229, Plate 27 (discussion of variation; color plate; range, in part). Etnier and Starnes, 1993:310, 312-313 (key, in part; description, in part; Tennessee range, in part;

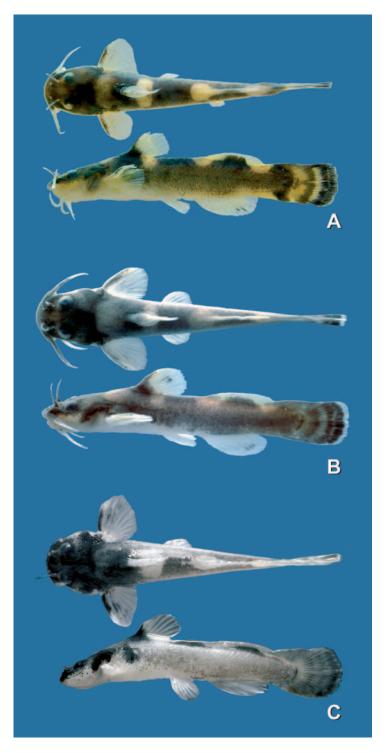


Fig. 2. (A) *Noturus fasciatus*, 47 mm SL, MOSU 2228, Tennessee, Hickman Co., Brushy Fork Creek. Photograph by D. J. and L. V. Eisenhour. (B) *Noturus elegans*, 44 mm SL, MOSU 1296, Kentucky, Casey Co., Green River. Photograph by D. J. and L. V. Eisenhour. (C) *Noturus crypticus*, 54 mm SL, SIUC 23165, Tennessee, Greene Co., Little Chucky Creek. Photograph by B. M. Burr.

discussion of variation). Mettee et al., 1996:374, 392–393 (in key, in part; description, in part; color plate from Duck River system, Tennessee). Etnier, 1997:98 (imperiled, in part; *N. elegans* is a complex of species). Warren et al., 1997:150 (range, in part). Boschung and Mayden, 2004:337–338 (description, in part; color image represents *N. fasciatus*).

- *Noturus (Rabida)* sp.: Eisenhour et al., 1996:41–46 (distribution, habitat, and conservation status review).
- Noturus sp. cf. N. elegans: Grady and LeGrande, 1992:750, 751, 754–758, 761, 763, 765–766, 768 (N. elegans-like populations from Duck and lower Tennessee rivers are taxonomically distinct; phylogenetic relationships; range map and biogeography). Etnier and Starnes, 1991:130 (jeopardized in Tennessee). Warren et al., 1997:151 (range; candidate for listing).
- *Noturus* sp. cf. *elegans* 2. saddled madtom: Warren et al., 2000:21 (vulnerable).
- N. aff. elegans: Hardman, 2004:397, 400, 401, 402, 406 (in phylogeny; also as N. (R). aff. elegans; probably related to N. elegans, N. hildebrandi, and N. baileyi).

*Holotype.*—SIUC 52378, 62.4 mm SL, Tennessee, Hickman County, Brushy Fork Creek, off Brushy Road, 1.6 km W of junction with Tennessee Highway 48, N of Aetna, 35°40'25"N, 87°31'03"W, B. M. Burr, C. A. Taylor, and K. M. Cook, 1 Nov. 1992.

Paratypes.—SIUC 20514, 3, 30.0-44.5 mm SL, Tennessee, Hickman County, Brushy Fork Creek, off Brushy Road, 1.6 km W of junction with Tennessee Highway 48, N of Aetna, 35°40'25"N, 87°31'03"W, B. M. Burr, C. A. Taylor, and K. M. Cook, 1 Nov. 1992. USNM 230594, 50, 29.3-51.5 mm SL; INHS 98366, 10, 28.2-54.4 mm SL; SIUC 52381, 10, 38.0-67.6 mm SL; UAIC 13875.01, 10, 32.2-63.5 mm SL; UT 48.1207, 10, 33.0-53.5 mm SL; UMMZ 243663, 10, 34.6-53.4 mm SL; TU 196774, 10, 32.0-66.2 mm SL; UF 143185, 10, 25.4–59.5 mm SL; Morehead State University, MOSU 2069, 10, 32.5-60.0 mm SL, Tennessee, Hickman County, Brushy Fork Creek, off Tennessee Highway 48, N of Aetna, 35°40'25"N, 87°31'03"W, R. W. Bouchard, 29 Nov. 1969.

*Non-type material.*—(Numbers in parentheses are numbers of specimens measured followed by the number used for meristic data.) Tennessee: UT 48.299 (0, 2), Bedford Co., Duck R., 9.1 km NW Shelbyville, 3.2 km NW Elbethel, 20 May 1978. USNM 230594 (5, 10), Hickman Co., Brushy Fork Cr., off TN 48, N of Aetna, 29 Nov. 1969. SIUC 3635 (0, 7), Hickman Co., Trib., Duck R., off TN 48, 4.8 km N Kimmins, 6 Sept. 1981. SIUC 19677 (3, 0), Hickman Co., Cane Cr., off Cane Creek Road, 0.8 km S Pleasantville, 22 May 1992. SIUC 19677 (0, 4), Hickman Co., Cane Creek, 0.8 km S Pleasantville, 22 May 1992. USNM 230521 (0, 5), Hickman Co., Piney Fork Cr., at TN 48, 28 Nov. 1969. SIUC 20713 (2, 0), Hickman Co., Beaverdam Cr., at TN 100W bridge, 8 km SSW Centerville, 1 Nov. 1992. SIUC 15188 (0, 6), Hickman Co., Beaverdam Cr., at TN 48, just N of Aetna, 21 May 1987. USNM 201387 (0, 5), Hickman Co., Beaverdam Cr., at TN 50 crossing, E edge of Coble, 17 May 1965. SIUC 20514 (2, 0), Hickman Co., Brushy Fork Cr., off Brushy Road, 1.6 km W junction with TN 48, N of Aetna, 1 Nov. 1992. SIUC 20496 (1, 0), Humphreys Co., Hurricane Cr., off Hurricane Creek Road, 9.7 km S Waverly, 2 Nov. 1992. SIUC 20496 (0, 6), Humphreys Co., Hurricane Cr., 9.6 km S Waverly, 2 Nov. 1992. UT 48.119 (0, 11), Humphreys Co., Hurricane Cr., ca. 9.6 km E of co. rd. 6222, 20 Sept. 1972. UT 48.144 (0, 10), Humphreys Co., Buffalo R., 2.0 river km above mouth, 10 Nov. 1973. SIUC 3357 (0, 4), Humphreys Co., Tumbling Cr., ca. 11.2 km ENE Bucksnort, 27 April 1981. USNM 264851 (0, 6), Lewis Co., Little Swan Cr., in Meriweather Lewis National Monument, 16 March 1972. SIUC 20148 (1, 0), Lewis Co., Big Swan Cr., TN 99 bridge, 23 July 1992. SIUC 20801 (2, 2), Lewis Co., East Fork Cane Cr., off Cane Road, 7.2 km NNW Hohenwald, 1 Nov. 1992. UT 48.343 (0, 9), Lewis Co., Little Swan Cr., 200 m above Natchez Trace Parkway crossing, 10.4 km ESE Hohenwald, 20 March 1979. SIUC 5993 (0, 2), Lewis Co., Swan Cr., 0.2 mi. E Gordonsburg, 5 Nov. 1982. UT 48.313 (0, 1), Marshall Co., Duck R., 3.7 river km upstream of US 31A crossing, ca. 3.6 km SE Chapel Hill, 5 Nov. 1978. SIUC 11160 (0, 1), Marshall Co., Duck R., in Henry Horton State Park, 14 Oct. 1980. UT 48.117 (0, 1), Maury Co., Big Bigby Cr., above Stauffer Chemical Plant, 27 Sept. 1972. SIUC 20289 (1, 6), Perry Co., Cane Cr., TN 50 bridge, 3.2 km E Lobelville, 26 July 1992. TU 89560 (10, 0), Wayne Co., Fortyeight Cr., 12 km E of Waynesboro, US 64, 30 July 1974. SIUC 3599 (3, 8), same data as TU 89560, 6 Sept. 1981. SIUC 20254 (0, 2), Wayne Co., Buffalo R., 2.4 km ESE Little Hope, 25 July 1992. UT 48.148 (0, 8), Wayne Co., Moccasin Cr., 0.2 km W Natural Bridge, N of US 64, 10 Nov. 1973. SIUC 42985 (1, 1), Wayne Co., Weatherford Cr., 5.6 km upstream of Lutts, 27 March 1998.

*Diagnosis.*—Distinguished from other members of the *N. elegans* species group by having: 1)

Species/Stream	14	15	16	17	18	19	20	Ν	mean	SD
N. elegans										
Green R., KY	2	24	23	7				56	15.63	0.752
Barren R., KY	1	6	20	15	5			47	16.36	0.919
N. fasciatus										
Duck R., TN			3	25	29	5	3	65	17.69	0.865
N. crypticus										
Little Chucky Cr., TN		3	4	1				8	15.75	0.707
Dunn Cr., TN				1				1	17.00	_
Unassigned specimens of the $\Lambda$	. elegans com	nplex								
Piney Cr., AL	0	-	1					1	16.00	_
Flint R., AL			2					2	16.00	_
Paint Rock R., AL			1	1				2	16.50	0.707

 TABLE 1.
 FREQUENCY DISTRIBUTION, MEAN, AND STANDARD DEVIATION (SD) OF ANAL-FIN RAY COUNTS IN Noturus elegans, N. fasciatus, N. crypticus, AND UNASSIGNED SPECIMENS OF THE N. elegans COMPLEX.

a more elongate anal fin with modally 18 rays (vs. modally 16; Table 1); 2) a boldly contrasting pigment pattern of 3-4 ivory to yellow ellipses on the nape and dorsum interrupted by dark chocolate brown saddles (vs. pale blotches not strongly contrasting); 3) adipose fin blotch extending to middle of fin but nearly always to its edge (vs. blotch in lower half of fin or confined to base); 4) small, dark saddle on ventral edge of caudal peduncle (vs. dark saddle absent from caudal peduncle); 5) adipose fin rather high, intermediate in length and weakly connected to caudal fin (vs. low, long in length, and moderately connected to caudal fin in N. crypticus or moderate in height and length and well connected to caudal fin in N. elegans); 6) diffuse band or crescent-shaped dark blotch at caudal fin base (vs. dark crescent-shaped blotch absent at caudal fin base); 7) modally 36 post-Weberian vertebrae (vs. modally 34-35); 8) slender body shape, with body width at pectoral fin origin 21% or less of SL and shorter than anal fin base length (vs. body width 23% or more of SL and longer than anal fin base length in N. crypticus). Additionally, N. elegans and N. fasciatus differ by 6.6% sequence divergence (uncorrected p) for Cyt*b* and unique genotype distributions for eight allozyme loci: s-AAT-A, FUM-A, GPI-A, sICDH-A, LDH-B, MPI-A, Pep-B, and PGM-A (Table 2, [Grady, 1987; Grady and LeGrande, 1992]).

*Description.*—Body measurement data for 31 individuals are shown in Table 3. Dorsal rays 6, very rarely 5; anal rays 16–20 (modally 18); caudal rays 45–54 (modally 47 or 49); pectoral rays 8–9 (modally 9); pelvic rays 9–10 (modally 9); post-Weberian vertebrae 34–37 (modally 36), pre-operculomandibular pores 10–11 (modally 11); posterior pectoral spine serrae 7–8; internasal

pores 2 (very rarely 1); gill rakers 5–7 (modally 6). A slender madtom; body relatively narrow at pectoral fin origins, about 21% or less of SL; head narrow and flattened dorsally; pectoral, dorsal, and pelvic fins short and rounded; adipose fin rather high and relatively short, weakly connected to caudal fin. Pectoral spine relatively short and straight with 6–9 recurved posterior serrae and numerous, fine distinct

TABLE 2. GENOTYPE DISTRIBUTIONS FOR ALLOZYME LOCI THAT VARIED AMONG POPULATIONS OF THE N. elegans SPECIES GROUP. Presumptive alleles are designated alphabetically, with the most anodal allele as "a." Allelic designations are relative only to samples of the N. elegans species group. Sample size per genotype is indicated in parentheses.

Locus	N. elegans	N. fasciatus	N. crypticus
mAAT-A	bb (10)	bb (10)	aa (4)
sAAT-A	bb (10)	aa (10)	aa (4)
sACOH-A	aa (7)	bb (10)	cc (4)
	ab (3)		
AK-A	bb (9)	aa (9)	bb (4)
		bb (1)	
FUM-A	bb (10)	aa (10)	aa (4)
GPI-A	aa (10)	bb (10)	bb (4)
GPI-B	aa (3)	bb (10)	cc (4)
	ab (5)		
	bb (2)		
sICDH-A	aa (10)	bb (10)	aa (4)
LDH-B	bb (10)	aa (10)	cc (4)
SMDHP-A	aa (10)	aa (10)	bb (4)
MPI-A	bb (10)	aa (10)	bb (2)
			ab (2)
Рер-В	aa (10)	bb (10)	aa (4)
Pep-D	aa (10)	cc (5)	bb (4)
*		ac (5)	
PGM-A	cc (9)	aa (10)	bb (4)
	bc (1)		

	N. crypticus			N. elegans			N. fasciatus		
Measurement	mean	SD	range	mean	SD	range	mean	SD	range
1. Snout to occiput	25.3	1.7	22.5-27.6	23.8	1.1	21.1-26.3	23.2	1.1	21.4-26.4
2. Snout to dorsal-fin origin	38.6	1.7	35.4 - 40.9	36.0	1.2	33.6-38.3	34.2	1.8	31.0-38.
3. Snout to pectoral-fin origin	27.5	1.5	25.7 - 29.4	25.8	1.2	23.1 - 29.0	25.1	1.4	21.2-27.
4. Snout to pelvic-fin origin	47.1	0.6	32.7 - 50.4	47.5	1.2	45.5 - 49.6	45.9	1.6	43.4-49.
5. Occiput to dorsal-fin origin	3.7	1.0	12.1 - 15.2	12.6	0.8	11.3 - 14.1	11.0	1.0	9.1-13.
6. Pectoral-fin origin to dorsal-fin origin	21.4	1.9	18.9–24.4	8.6	1.1	16.3-20.4	16.7	1.3	14.7-20.
7. Pectoral-fin origin to pelvic-fin origin	26.8	1.2	24.8-28.1	25.6	1.7	21.5-28.6	23.9	1.4	21.7-26.
<ol> <li>B. Dorsal-fin origin to pelvic-fin origin</li> </ol>	24.1	1.7	21.6-27.0	24.1	2.1	19.9–29.3	23.2	1.7	21.3–28.
9. Dorsal-fin insertion to pelvic-fin origin	17.1	2.3	14.2–21.4	17.9	2.1	15.4-23.3	17.0	1.8	14.9-22.3
10. Pelvic-fin origin to adipose-fin origin	24.5	1.6	21.8-26.8	24.1	1.4	21.9-28.0	25.4	1.2	22.5–27.9
<ol> <li>Pelvic-fin origin to anal-fin origin</li> </ol>	15.2	1.2	12.9–16.7	14.9	1.1	13.1–17.1	15.0	1.5	11.2–18.0
12. Anal-fin origin to adipose-fin origin	17.2	1.4	15.6–19.6	17.4	1.0	15.5–19.0	18.2	0.9	16.2–20.
13. Anal-fin origin to dorsal-fin insertion	24.3	1.4	22.1-26.1	24.8	1.5	21.1-27.9	25.3	0.9	23.7–27.
14. Dorsal-fin insertion to adipose-fin origin	20.0	1.3	17.7–21.9	19.9	2.2	15.8-24.0	22.8	1.7	18.7–25.
15. Adipose-fin origin to anal-fin insertion	24.5	0.9	22.6-25.3	24.6	1.4	21.7-27.4	22.4	1.1	19.7–24.
16. Adipose-fin base length	25.6	1.0	24.0 - 26.9	27.3	1.9	24.5 - 31.9	24.3	1.6	20.5-27.4
17. Anal-fin origin to adipose-fin insertion	31.5	1.0	29.7–32.5	32.5	1.2	30.5-35.2	32.5	1.2	29.7–35.
18. Anal-fin insertion to adipose-fin insertion	15.1	1.2	13.8–17.0	14.8	0.8	13.0-15.9	14.8	0.7	13.1–16.
19. Anal-fin base length	21.8	0.7	20.7 - 22.7	21.8	1.4	19.4 - 24.4	21.7	1.1	19.4-24.2
20. Dorsal-fin base length	12.9	0.7	12.1 - 14.3	11.8	0.7	10.2 - 13.2	11.0	0.8	9.4-13.
21. Occiput to pectoral-fin origin	15.5	1.0	13.8–17.4	13.6	1.0	11.9–16.7	12.9	1.0	11.3–15.
22. Least fleshy interorbital width	8.1	0.8	6.8–9.4	7.6	0.6	6.5-8.8	7.4	1.0	5.8–9.8
23. Body width at pectoral-fin origin	24.1	0.8	22.7-25.0	20.0	1.0	18.5–22.2	18.9	1.0	16.9–21.
24. Standard length (in mm)	56.8	5.9	45.9 - 64.7	49.2	6.4	36.0 - 61.7	52.0	7.6	39.5-67.
25. Body width under dorsal-fin origin	19.0	1.4	15.8-20.4	15.9	2.2	12.5-21.9	14.4	1.8	12.0–20.
26. Head width	25.1	1.3	23.2 - 26.4	21.7	0.9	20.2 - 23.4	20.7	1.0	18.5 - 22.
27. Pectoral spine length 28. Dorsal spine length	14.0 8.2	$0.9 \\ 1.0$	12.9-15.6 6.6-9.5	$12.9 \\ 10.3$	$0.9 \\ 1.2$	11.2–14.9 8.6–12.6	12.8 9.8	$0.9 \\ 1.0$	11.0–15. 8.0–14.

 TABLE 3.
 PROPORTIONAL MEASUREMENTS OF EIGHT Noturus crypticus, 30 N. elegans, AND 31 N. fasciatus EXPRESSED AS

 PERCENT OF SL. Numbered measurements correspond with those of Fig. 1.

serrae anteriorly (Fig. 3). Sexual dimorphism observed in a few pairs from late May to late July. During this presumed nesting season males have swollen or enlarged lips and adductor mandibulae muscles behind the eyes; the abdomens of ripe females are swollen with eggs during the nesting season. Largest individual is 72 mm SL (85 mm TL) from Fortyeight Creek, Wayne County, Tennessee (TU 89560). *Coloration in life.*—One of the most striking of madtom species when seen alive, especially in clear, spring-fed streams in the lower Duck River system (e.g., Beaverdam, Brushy Fork, and Cane creeks). The clearly demarcated saddles are chocolate brown to black, interspersed with ivory to light yellow ellipses. *Noturus elegans* does not develop unique color patterns during the nesting season, but remain boldly

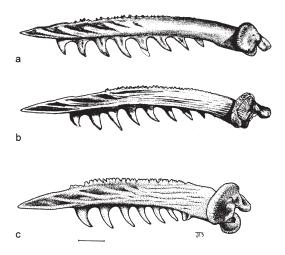


Fig. 3. Left pectoral spines of: (a) Noturus fasciatus, USNM 201600, 59 mm SL; (b) N. elegans, UMMZ 155526, 52 mm SL; and (c) N. crypticus, SIUC 23165, 59 mm SL. Figures (a) and (b) drawn by W. L. Brudon in Taylor (1969); (c) by J. T. Sipiorski, SIUC.

marked throughout the year and from young to adult life stages.

Coloration in alcohol.-Most individuals boldly marked with four dark saddles over the dorsum interspersed with rectangular or elliptical white to yellowish blotches resulting in a striking color pattern (Fig. 2A). First dark saddle at dorsal fin, second dorsal to pelvic fins, third at middle of adipose fin, fourth immediately anterior of caudal fin. In most individuals dark saddles reach axial streak, or nearly so, and often connect with saddles to the anterior or posterior. White to vellowish ellipses between dark saddles usually not as wide as saddles and rarely continue ventrally to axial streak. First light blotch or ellipse on nape, second just posterior to dorsal fin, third immediately in front of adipose fin, and fourth on posterior end of adipose fin. Top of head is dark brown, branchiostegal rays and belly immaculate. Side of the body tan to dark brown and fades in intensity toward venter. Dark blotch or crescent-shaped bar present at base of caudal fin followed by a light crescent-shaped blotch on base of caudal rays. Remainder of caudal fin varies from uniform brown to usually with three diffuse streaks interspersed with lighter streaks; margin of caudal fin may be clear. Dorsal fin dark brown at anterior base and splotches of dark pigment can occur on tips of dorsal rays. Pectoral fins have dark brown on skin covering the spine and several rays; pelvic fins immaculate. Anal fin, especially in small individuals, immaculate, but adults often have dark brown pigment at base and on proximal edge of posterior rays. Adipose fin blotch reaches middle of fin and most often continues to margin. Nasal barbels brown, all others whitish or nearly so.

Variation.-Morphological variation is mostly limited to pigmentation and is most conspicuous in the contrast between dark saddles and light intervening areas. Individuals from mainstem rivers, particularly the Duck and Buffalo rivers, or other sites affected by turbidity from agricultural runoff or major rain events are more somber in color and the light ellipses so distinctive of clear tributary streams are somewhat obscured. Variability in other pigmentation features is prominent at the pectoral fin base, in the anal fin, and in the adipose fin (Fig. 4). A disturbing aspect of this study was the discovery that specimens collected about 15 years ago and that were exposed to fluorescent and incandescent light have bleached so completely that no pigmentation is visible under a standard microscope. Had the specimens not been identified soon after collection, it would be nearly impossible to accurately identify them now.

Finally, four specimens from the mainstem Duck River in Henry (SIUC 11160), Marshall (UT 48.313), and Bedford (UT 48.299) counties, Tennessee, have more subdued saddles than typical *Noturus fasciatus* (Etnier and Starnes, 1993). These specimens also have fewer anal rays (15–17) than typical *N. elegans*, but typical pelvic ray counts (9–9), and a dark band that extends to the margin of the adipose fin. Because intensity of the dorsal saddles varies in *N. fasciatus* and the specimens with the boldest saddles come from the clearest streams, the more subdued pigmentation may be correlated to higher turbidity of the Duck River mainstem, and we treat these specimens as *N. fasciatus*.

Comparisons .- Noturus fasciatus is one of 18 species of the subgenus Rabida that are characterized by: 1) the presence of serrae (dentations) on both the anterior and posterior edges of the pectoral spine; 2) body relatively short and usually chunky, although elongate in some species; 3) lower jaw included in upper jaw; 4) anal fin with 12-19 rays; 5) vertebrae 30-39; 6) usually 11 preoperculomandibular pores; 7) usually 9 pelvic rays; 8) usually 8 pectoral rays; 9) caudal rays relatively short, usually 15-19 branched rays. All species are mottled, or covered with irregular dark blotches or saddles. The background color of the body may be pinkish, yellowish, or brownish, becoming darker with age (Taylor, 1969).

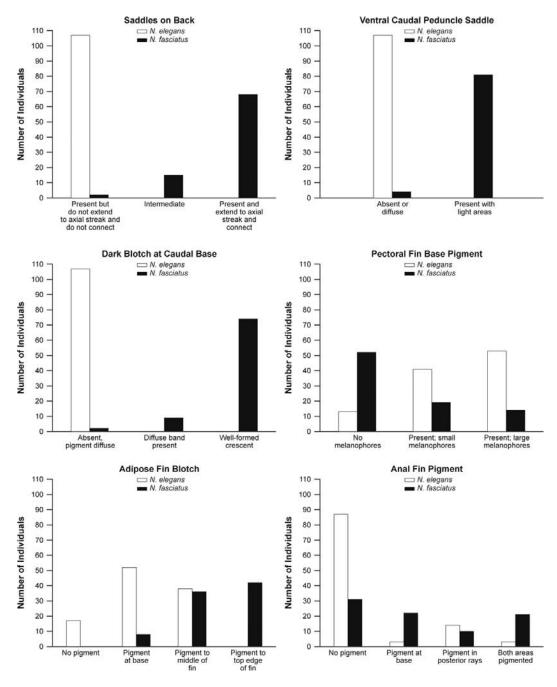


Fig. 4. Comparison of six scores of pigmentation characters for Noturus elegans and N. fasciatus.

*Noturus fasciatus* has been most frequently confused with or considered conspecific with *Noturus elegans* (Fig. 2B). The latter species differs from *N. fasciatus* (Table 1) in having usually 14–18, modally 16, anal rays (vs. 16–20, modally 18, anal rays); saddles on back not prominent and not extending to axial streak (vs. bold dark saddles that extend to axial streak); adipose fin

blotch extending to base or middle of fin only (vs. adipose blotch extending to middle of fin and most often to its margin); no ventral caudal peduncle saddle (vs. a small saddle with tiny light blotches in front and behind); no dark blotch or crescent at base of caudal fin (vs. diffuse band or well formed crescent); adipose fin well connected to caudal fin (vs. weakly connected to

Character	N. elegans	N. fasciatus	N. crypticus
Size (SL, TL)	62, 74	72, 85	65, 76
Adipose fin blotch	brown, extends to middle of fin, but just as often only at base	chocolate brown or black, typically extends to middle of fin or margin	blackish, extends into lower half of fin only
Saddles on back	present, but not prominent; do not extend to axial streak and do not connect to one another	present and prominent; extend to axial streak and connect to one another	present as blotches along dorsal ridge only
Ventral caudal peduncle saddle	absent	present with tiny light blotches in front and behind	absent
Dark blotch at caudal base	absent	diffuse band to well formed crescent	absent
Color pattern of body	somber, blotches and saddles not prominent	boldly marked with distinctive dark saddles and blotches	dark blotches confined to dorsal ridge
Large melanophores on cheek	absent	absent	prominent below and behind eye
Adipose fin in adults	moderate in height and length; well connected to caudal fin	high, intermediate in length; weakly connected to caudal fin	low, relatively long; moderately connected to caudal fin
Lateral body pigment	uniform light to dark brown on side	chocolate brown fading to much lighter on lower side	plain light gray with scattered large melanophores on side
Anal rays	14–18, modally 16	16–20, modally 18	15–17, modally 16
Post-Weberian vertebrae	34–36, modally 35	34–37, modally 36	32–35, modally 34
Pelvic rays	8-10, modally 9	9–10, modally 9	8–9, modally 8
Body width at pectoral fin origin	19–22% of SL	17–21% of SL	23–25% of SL
Relationship of anal fin base length to body width at pectoral fin origin	anal base longer than or equal to body width	anal base longer than or equal to body width	anal base shorter than body width

TABLE 4. COMPARISON OF SPECIES OF THE PRESUMED *elegans* GROUP OF *Noturus*.

caudal fin); modally 35 vertebrae (vs. modally 36 vertebrae). Noturus elegans reaches a maximum SL of about 62 mm (vs. about 72 mm SL in N. fasciatus). Noturus crypticus differs from N. fasciatus in having the adipose fin blotch extending only into the lower half of the fin, in lacking true saddles on the back and only dark blotches along the dorsal ridge, in lacking a ventral caudal peduncle saddle and dark blotch at the caudal base, in having modally 16 anal rays and modally 34 vertebrae, and in having a chunkier body (Table 4).

Quantifying body shape is useful in distinguishing species of the Elegant Madtom species group. Sheared PCA separated *Noturus fasciatus* from *N. crypticus* in multivariate space; moderate overlap was present between clusters of *N. elegans* and *N. fasciatus* (Fig. 5). Most discrimination occurred along the sheared PC 2 axis; loadings for *N. fasciatus* were highest for a set of variables associated with a less robust body, a longer dorsal spine, and a more anteriorly placed dorsal fin than *N. elegans* or *N. crypticus*. Univariate analysis of the morphometric variables revealed that *N. fasciatus* differs from *N. crypticus* in having a narrower head (19–23% SL vs. 24–26% SL) and a narrower body at the pectoral fin origins (19–22% SL vs. 23–25% SL; Table 3).

Distribution.—Noturus fasciatus occurs in the Duck River system and adjacent western tributaries of the Tennessee River in Hardin and Wayne counties, Tennessee (Fig. 6). It is known historically from tributaries of the lower Duck and Buffalo rivers but from only a few records from the mainstem Duck River in Bedford, Henry, and Marshall counties. The species also is recorded from the lower arm of the Tennessee River

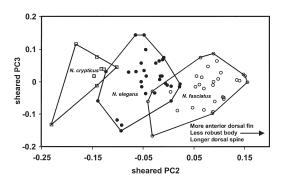


Fig. 5. Morphometric scores on sheared PC axes 2 and 3 for eight *Noturus crypticus*, 30 *N. elegans*, and 31 *N. fasciatus*.

drainage, historically occurring at five localities in the Indian Creek system and one locality in Rogers Creek (Horse Creek subsystem).

Museum records and other sampling data record 59 collections of *Noturus fasciatus* from 33 localities. *Noturus fasciatus* was taken at 13 of 33 sites in recent collections (Eisenhour et al., 1996). Five records were from small tributaries of the lower Duck River, and seven were from the Buffalo River subsystem. Only one specimen was collected from the Duck River mainstem. The maximum number of individuals collected per site during the day was six and the mean was 2.1. Sixteen historical collections of *N. fasciatus* are from the Buffalo River system in Lewis County; however, no specimens were collected from this area in our survey and suitable habitat was not present at additional historical localities reconnoitered (but not sampled) in the upper Buffalo River subsystem. There are recent (post-1998) records from the Indian Creek system (e.g., SIUC 42985) and from the Horse Creek subsystem (UT 48.1195).

Habitat and ecology.—In recent collections (1993–2004), all Noturus fasciatus were taken in riffle habitats with gravel, cobble, rubble, or slate substrate. Mean depth was 0.2 m (range 0.1–0.3 m), and stream width averaged 13.6 m (range 8–25 m). Mean current readings from three capture sites ranged from 0.59–0.76 m/sec (grand mean 0.66 m/sec). Riffles are typically occupied during daylight hours when individuals likely are buried within the interstices of gravel. (It took repeated riffle kicks and deep disturbance of gravel to dislodge individuals.) Although nocturnal observations are limited, the Saddled Madtom ventures into pools and pool

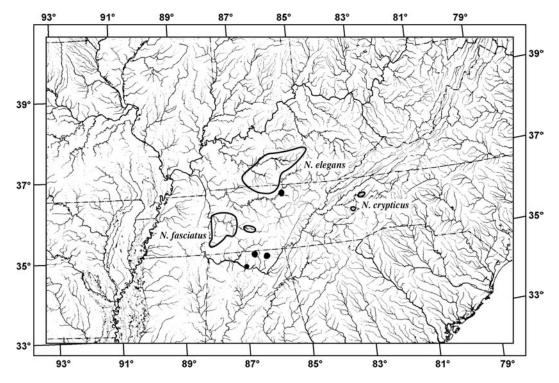


Fig. 6. Geographic range of *Noturus elegans*, *N. fasciatus*, and *N. crypticus*. Black spots represent localities where specimens that appear to be close relatives of or conspecific with members of the *Noturus elegans* species group have been taken. See text for details.

margins to forage at night or during crepuscular periods. As judged from its small adult size (to 72 mm SL), N. fasciatus probably lives no longer than 2-3 years (see review of length-age relationships of other madtom species in Burr and Stoeckel [1999]). Preserved females contain mature oocytes from late May to late July; females are mature at sizes as small as 44 mm SL (B. M. Burr et al., pers. obs.). In recent surveys, N. fasciatus was frequently associated with the Largescale Stoneroller (Campostoma oligolepis), Rosyside Dace (Clinostomus funduloides), Tennessee Shiner (Notropis leuciodus), Banded Sculpin (Cottus carolinae), Fantail Darter (Etheostoma flabellare), Saffron Darter (E. flavum), and Redline Darter (E. rufilineatum).

In general, critical habitat for *Noturus fasciatus* includes second and third order streams with clear water, dark gravel and slabrock substrates, and abundant riffle habitat. Beyond habitat affinity, little is known of the ecology of this species. Nesting habitat is probably similar to that of the closely related *N. elegans* (i.e., pools or slow raceways that contain slabrocks above and below riffles [Burr and Dimmick, 1981]).

Conservation status.-Historical collections contain 540 specimens, or a mean of 9.3 individuals per collection. Excluding two very large samples (TU 89560 [n = 81] and USNM 230594 [n =125]), the mean specimens per collection is 6.0. The few sites from which we obtained specimens and the low number of specimens per site (mean = 2.1) indicate that the species has either: 1) declined dramatically in abundance and range over the past 10-20 years; or 2) our seasonal timing, collecting techniques, and efforts differ significantly relative to those of previous collectors. Low densities of Noturus fasciatus in recent surveys might reflect our diurnal sampling of a nocturnal species. Most madtoms are more vulnerable to standard collecting methods at night (Etnier and Starnes, 1993). Field data indicating time of collection are not available for the majority of historical collections, precluding direct comparison with recent collections. Nonetheless, repeated diurnal collections by BMB have consistently yielded ten or more N. fasciatus from several localities in the previous 15 years, suggesting that the species has disappeared from some sites over the past 10-20 years.

Factors contributing to range decline of *Noturus fasciatus* were not investigated; however, severe drought in the late 1980s could have contributed to local extirpation. Peak spawning for *N. fasciatus* is probably in June and July, similar to related madtoms (Burr and Stoeckel, 1999). Low flows during the spawning period

might have disrupted nesting and reduced recruitment, especially in smaller tributaries lacking permanent spring input. Presently, the largest populations are in the Buffalo River subsystem where streams supplied by perennial springs are common.

The population in the mainstem Duck River would likely be extirpated by impounded water that would be created by completion of the Columbia Dam project, as has been proposed. Populations in tributaries of the lower Duck River system would not be dramatically affected. A number of potential threats, including channelization, removal of riparian vegetation, and agricultural runoff, all common occurrences in eastern North American streams, would result in an overall decrease in water quality. These disturbances have the greatest impact on small, high quality streams, critical habitat of Noturus fasciatus. Additionally, the wide variety of complex organic chemicals added to streams may interfere with the highly developed olfactory sense of madtoms, disrupting behavioral patterns important for survival (Etnier and Jenkins, 1980).

Other anthropogenic factors affecting *Noturus fasciatus* include bridge construction, which alters stream hydrology, modifies substrates, and contributes to removal of nesting habitat. During recent status surveys, bridge construction was observed at several historical localities in the Buffalo River subsystem. Neither suitable habitat for nor specimens of *N. fasciatus* were recorded at these sites. Although bridge construction impacts a relatively small area, it could contribute to extirpation of small, disjunct populations and further fragmentation of the range of the species.

*Noturus fasciatus* should be included among the rare animals for the State of Tennessee. Management should include educating landowners of the importance of maintaining riparian vegetation and controlling erosion and agricultural waste along streams.

*Etymology.*—The specific epithet, *fasciatus*, is a Latin word meaning to "envelop with bands" (Brown, 1956) and is in reference to the striking bands or saddles on the upper half of the body. The common name emphasizes the same pattern.

Noturus crypticus, new species Chucky Madtom Figure 2C

Noturus elegans Taylor, 1969:155 (description of Dunn Creek, Tennessee, specimens and re-

lated data). Etnier and Starnes, 1993:310, 313 (discussion of specimens from Dunn and Chucky creeks). Mettee et al., 1996:374, 392– 393 (in key, in part; description, in part). Boschung and Mayden, 2004:337–338 (description, in part).

- *Noturus* sp. cf. *elegans*: Warren et al., 1997:151 (range; candidate for listing).
- *Noturus* sp. cf. *elegans* 1: Warren et al., 2000:21 (in part, threatened in the southern United States).

*Holotype.*—SIUC 52377, 60.6 mm SL, Tennessee, Greene County, Little Chucky Creek at mouth of Jackson Branch, 36°07'11"N, 83°02'04"W, B. M. Burr, D. J. Eisenhour, and K. R. Piller, 4 Sept. 1994.

*Paratypes.*—SIUC 52379, 1, 64.7 mm SL, Tennessee, Greene County, Little Chucky Creek at mouth of Jackson Branch,  $36^{\circ}07'11''N$ ,  $83^{\circ}02'04''W$ . UAIC 12430.01, 1, 48.7 mm SL; SIUC 52380, 3, 54.0–58.7 mm SL; UT 48.724, 2, 45.9–56.0 mm SL; Tennessee, Greene County, Little Chucky Creek, 80 m upstream of Bible Bridge Road crossing, 3.4 km E of Warrensburg,  $36^{\circ}07'27''N$ ,  $83^{\circ}03'08''W$ .

*Non-type material.*—UMMZ 131386, 1, Tennessee, Sevier County, Dunn Creek, Jones Cove at mouth of Yellow Breeches Creek.

Diagnosis.—Distinguished from other members of the Noturus elegans species group by having: 1) modally 16 anal rays (vs. modally 18 anal rays in N. fasciatus; Table 1); 2) modally 8 pelvic rays (vs. modally 9 pelvic rays); 3) 2-10 medium-large sized melanophores on the cheek (vs. large melanophores absent on cheek); 4) adipose fin band extending only to base or half-way up fin (vs. extending half-way up to edge of fin in N. fasciatus); 5) adipose and caudal fins distinctly joined at their juncture (vs. weakly connected in N. fasciatus); 6) three evenly spaced pale and dark bands in caudal fin (vs. caudal fin bands usually two and unevenly spaced); 7) robust body shape, with body width at pectoral fin origin 23% or more of SL and greater than anal fin base length (vs. slender body shape, with body width at pectoral fin origin 22% or less of SL and less than anal fin base length). Absence of shared alleles for eight allozyme loci (mAAT-A, sACOH-A, GPI-B, LDH-B, sMDHP-A, Pep-b, Pep-D, and PGM-A) distinguishes N. crypticus and N. fasciatus, which also differ by 5% Cytb sequence divergence. Similarly, N. crypticus and N. elegans share no alleles at eight allozyme loci (mAAT-A, sAAT-A, sACOH-A, FUM-A, GPI-A, GPI-b, LDH-B, sMDHP-A, and Pep-D [Table 2]) and are 6.3% divergent for Cyt*b*.

Description.-Body measurement data for 31 individuals are shown in Table 4. Dorsal rays 6; anal rays 15-17 (modally 16); caudal rays 42-51 (mean 46.7); pectoral rays 7-9 (modally 8); pelvic rays 8-9 (modally 8); post-Weberian vertebrae 32-35 (modally 34); preoperculomandibular pores 10-12 (modally 11); posterior pectoral spine serrae 7-8; internasal pores 1-2 (modally 2); gill rakers 4-7 (modally 5). A robust madtom; body wide at pectoral fin origins, greater than 23% of SL; head wide and flattened dorsally; pectoral and dorsal fins short and rounded; adipose fin low and well-connected to caudal fin. Pectoral spine relatively short, stout, and slightly curved with 6-8 recurved posterior serrae and numerous, fine, distinct, anterior serrae (Fig. 3). Sexual dimorphism apparent only in a pair of specimens (UT 48.724) collected 1 May; the male has enlarged adductor mandibulae muscles immediately posterior to the eyes and the female has a distended abdomen. Largest individual is a 64.7 mm SL (74 mm TL) specimen from Little Chucky Creek (SIUC 52379).

Coloration.—The most distinctive pigmentation is on the cheek, dorsum, and fins (Fig. 2C). A few (2-10) medium-sized melanophores present on cheek below and behind eye. Otherwise head pale on venter and sides and dark on dorsum, with an extension of dark pigmentation about halfway down operculum. Three dark, nearly black, blotches on dorsum, ending abruptly above lateral midline of body. First blotch at dorsal fin, second blotch immediately anterior to the leading edge of adipose fin, and third blotch at middle base of the adipose fin. Anterior to each of these dark saddles is oval, pale saddle. These blotches only a little paler than sides of body, and pale and dark blotches only moderately contrasting. Pigmentation along sides moderate in intensity and slightly concentrated along myosepta, creating faint chevrons. Belly anterior to pelvic fins lacks melanophores. Pigmentation at posterior dark dorsal saddle extends about halfway up adipose fin. Caudal fin usually with three broad, evenly spaced, pale and dark contrasting bands and clear, narrow marginal band. Anal fin clear, or with some dark pigmentation on middle portions of rays. Pelvic fins clear and pectoral fins mostly clear, with dark pigment on spine and middle portions of first 2-3 rays.

Variation.—Identification of several specimens, all of which have been tentatively assigned to the

*Noturus elegans* species group (Taylor, 1969; Etnier and Starnes, 1993; Boschung and Mayden, 2004) has been problematic. A specimen (UMMZ 131386) from Dunn Creek in eastern Tennessee appears to be *N. crypticus*. This specimen is faded, but has dorsal pigmentation consistent with Little Chucky Creek *N. crypticus*, including prominent cheek melanophores, 9–8 pelvic rays, and 17 anal rays.

Six specimens from tributaries of the southern bend of the Tennessee River in Alabama (Pinev Creek [UMMZ 165877], West Fork Flint River [UT 48.8, UT 48.264], and Paint Rock River [SIUC 15528; Feeman, 1987]) are faded or have been cleared-and-stained and are not suitable for pigmentation comparisons. Earlier descriptions (Etnier and Starnes, 1993) and a photograph (Plate 13, Figure 3 in Taylor [1969]) indicate subdued dorsal saddles. Body shape is moderately chunky, and body width at bases of pectoral fin to SL percentages are 20.6-23.2, intermediate of that of Noturus crypticus and N. fasciatus. They differ from N. crypticus in lacking the distinctive cheek melanophores and having 9-9 pelvic rays; but have anal rays counts (Table 1) typical of N. crypticus. Available data are insufficient to determine whether these morphologically divergent specimens are geographic variants within N. crypticus or an undescribed taxon within the N. elegans species group. Repeated efforts to collect additional madtoms from these historical locations have been unsuccessful.

Taylor (1969) assigned four juveniles (UMMZ 168262) from the Roaring River (Cumberland River drainage) in Jackson County, Tennessee, to Noturus elegans. These specimens differ from the N. elegans species group in having fewer anal rays (modally 14), fewer vertebrae (modally 34), and more pelvic and pectoral rays (9). However, Taylor reported 11 preoperculomandibular pores for each specimen, and the pigment pattern and adipose-fin shape is more suggestive of the N. elegans species group. These represent the only collection of the N. elegans species group from the Cumberland River drainage. Again, these specimens are tentatively placed within the N. elegans species group, with no assignment to species. The four locations of these problematic specimens are included in the range map for the N. elegans species group (Fig. 6).

*Comparisons.*—*Noturus baileyi*, the Smoky Madtom, also is known from eastern Tennessee and is superficially similar, but differs in having a shorter anal fin with only 12–13 anal rays (modally 16 in *N. crypticus*), nearly lacking anterior serrae on the pectoral fin spines, and nearly lacking dorsal saddles. In pigmentation, *N.* 

crypticus differs from other members of the N. elegans species group in having medium-sized melanophores on the cheek and distinctive caudal fin pigmentation (Table 4). It further differs from N. fasciatus in having subdued dorsal saddles (bold in N. fasciatus) and adipose fin pigmentation only at the base or half-way up the fin (extending half-way or all the way up in N. fasciatus; Fig. 2). In N. crypticus, anal ray counts are modally higher (Table 1), there are usually eight pelvic rays on at least one side (usually 9-9 in other members of the species group), and the adipose and caudal fins are distinctly joined. Sheared PCA separated N. crypticus from N. fasciatus and nearly from N. elegans in multivariate space (Fig. 5). Most discrimination occurred along the sheared PC 2 axis, with loadings indicating that N. crypticus has a relatively posterior dorsal fin, robust body, and short dorsal fin spine compared to the other members of the species complex. *Noturus crypticus* has a relatively wide body (Fig. 2C); body width at pectoral fin origin is 23-25% of SL in N. crypticus and 17-22% of standard length in N. elegans and N. fasciatus (Fig. 7).

Distribution.—The Chucky Madtom is known from only two streams, both of the French Broad River system of eastern Tennessee (Fig. 6). It is known from a single specimen from Dunn Creek, Sevier County, and from about three stream km of Little Chucky Creek from the mouth of Jackson Branch downstream to Bible Bridge road crossing, Greene County. Surveys targeting the Chucky Madtom (Burr and Eisenhour, pers. obs.; Shute et al., pers. comm.; Lang et al., pers. comm.) in neighboring streams with potentially suitable habitat did not yield additional specimens.

Habitat and ecology.-Little Chucky Creek, the location of the only known extant population, is a small, moderate gradient tributary to the Nolichucky River, in the Ridge and Valley physiographic province. The reach of Little Chucky Creek supporting this population is about 5-7 m wide, has alternating riffles, runs, and pools, and is a watershed used primarily for grazing. Although we observed some siltation, bank erosion was minimal, and the reach was well shaded by riparian vegetation. This stream section contained extant populations of the Cumberland Bean (Villosa trabalis), Rainbow (Villosa iris), Mountain Creekshell (Villosa vanuxemensis), and Tennessee Clubshell (Pleurobema oviforme). These mussels are indicators of high water quality (Cicerello and Schuster, 2003); V. trabalis is endangered and P. oviforme and V. vanuxemensis are considered of special concern (Williams et al., 1993). Suitable habitat for *N. crypticus* in Little Chucky Creek is patchy. Upstream of the Bible Bridge road crossing, madtoms were found above a bedrock riffle in a 10-m run bordered by a *Justicia* bed. The run was 19–29 cm (mean 23.4 cm) deep, with a substrate of slab rocks over pea-size flat gravel, and had moderate flow (average 22.6 cm/sec). Similar habitat was not observed in extensive sampling upstream and downstream of this reach and no *Noturus crypticus* were recovered.

Three areas of potential Noturus crypticus habitat were observed in about 300 m of stream at the mouth of Jackson Branch. Primary habitat again was slow riffles and runs with moderate flow (24.0 cm/sec) in shallow water (range 13-27 cm, mean 20.4 cm) over pea-size flat gravel intermixed with slab rock and cobble. In the most productive area, three madtoms were found in about 29 m<sup>2</sup>. Madtoms often group during fall and winter (the most productive sampling periods for N. crypticus) and may be more widely dispersed during the spring and summer. Therefore, dense concentrations of this fish should not be expected at any time. In our surveys, N. crypticus was frequently associated with the Central Stoneroller (Campostoma anomalum), Bigeye Chub (Hybopsis amblops), Striped Shiner (Luxilus chrysocephalus), Banded Sculpin (Cottus carolinae), Greenside Darter (Etheostoma blennioides), Redline Darter (E. rufilineatum), Stripetail Darter (E. kennicotti), and Banded Darter (E. zonale).

The 1 May 1991 specimens appear to be a male and female in prespawning condition, suggesting an early summer spawning season, similar to *Noturus elegans* (Burr and Dimmick, 1981). September collections contained only adults (46–65 mm SL) in postspawning condition. Because so few specimens are known presently, there is no information available on diet, sex ratio, recruitment, spawning requirements, and other aspects of life history.

*Conservation status.*—The Dunn Creek specimen was collected in 1940; additional surveys (P. W. Shute et al., pers. comm.) have not yielded additional specimens, and the species may be extirpated from this stream. Surveys (B. M. Burr and D. J. Eisenhour, pers. obs.; P. W. Shute et al., pers. comm.; N. Lang et al., pers. comm.) of streams with potentially suitable habitat in eastern Tennessee found *N. crypticus* only in a 3km reach of Little Chucky Creek. This area has been intensively sampled for madtoms, by seining and snorkeling, since 1991, but success

has been sporadic; only 14 specimens have been collected. In May 1991, two (UT 48.724) were collected by TVA personnel at Bible Bridge road crossing. In September 1994, we collected four specimens (three in SIUC 52380, one retained by [MG for genetic analysis) at Bible Bridge Road crossing, and five specimens (one each in SIUC 52377 and SIUC 52379, three for genetic analysis), at the mouth of Jackson Branch. Subsequent surveys targeting madtoms were unsuccessful (P. W. Shute et al., pers. comm.; N. Lang et al., pers. comm.), but a collection by B. Kuhajda and D. Neely in March 2001 yielded one specimen (UAIC 12430.01). Recently, Conservation Fisheries Inc. personnel collected two specimens in May 2004 (J. R. Shute, pers. comm.) that were brought live to their aquaculture facility in Knoxville, Tennessee, with the intent of initiating a captive breeding program.

The range of Noturus crypticus, like other madtoms of eastern Tennessee (e.g., N. baileyi and N. flavipinnis [Dinkins and Shute, 1996]) likely has declined. Currently it occupies a tiny range and exists at a very low density (probably less than 100 individuals). The occupied reach of Little Chucky Creek is not pristine, but existing habitat and the fish and mussel faunas suggest the stream has not been substantially altered by anthropogenic influences. Upstream of the occupied reach we observed greater agricultural impacts, including application of herbicides to remove riparian vegetation and bank erosion and siltation from cattle access to streams. The capacity of the system to absorb sediment without damage to the biotic community may have been reached. If the samples are indicative of population size, this species is vulnerable to a variety of anthropogenic and stochastic events (e.g., drought, disease, floods).

*Noturus crypticus* should be protected as a federally endangered species. Promoting agricultural practices that minimize siltation and restoring and protecting riparian zones throughout the Little Chucky Creek watershed would preserve critical *N. crypticus* habitat.

*Relationships.*—Despite repeated efforts to reconstruct the history of madtom catfish diversification, none of the available phylogenies includes all members of the *Noturus elegans* species group. Taylor (1969) recognized only *N. elegans*, inclusive of populations in the Green, Duck, Cumberland, and Tennessee rivers, and placed it in the subgenus *Rabida*. He included *N. elegans* (as conceived above) and *N. trautmani*, an endemic to the Scioto River, Ohio, in the *elegans* group. Specimens of *N. trautmani* were last collected in 1957 (Trautman, 1981). A phylogeny

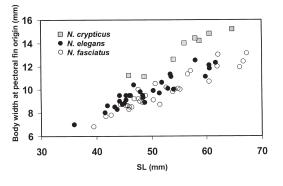


Fig. 7. Comparison of the relationship of body width at pectoral fin origin to SL for three species in the *Noturus elegans* species group.

based on karyological characters placed N. elegans in a clade with N. hildebrandi and N. albater (LeGrande, 1981). Using a combination of morphological, karyological, and allozymic characters and various phylogenetic inference strategies, Grady and LeGrande (1992) reconstructed seven madtom phylogenies that included N. elegans and N. fasciatus. The globally most parsimonious tree placed N. elegans and N. fasciatus in a clade with N. miurus and N. taylori, but other trees did not support a close relationship between N. elegans and N. fasciatus. The most recent comprehensive phylogenetic treatment of Noturus was based on mitochondrial and nuclear genes (Hardman, 2004) and generally supported N. elegans and N. fasciatus in a clade with N. baileyi and N. hildebrandi.

Parsimony and likelihood analyses of the partial Cytb data set (804 bp) generated trees that were consistent with Hardman's (2004) analysis of the complete Cytb sequence. A strict consensus of the 35 equally parsimonious reconstructions and the two best likelihood trees recognize N. crypticus and N. fasciatus as sisters and closely related to N. elegans, N. baileyi, and N. hildebrandi. However, parsimony trees place the N. crypticus–N. fasciatus lineage as basal to a N. elegans (N. baileyi + N. hildebrandi) clade, whereas likelihood trees recognize a N. elegans (N. crypticus + N. fasciatus) clade that is sister to N. baileyi + N. hildebrandi.

*Etymology.*—The specific epithet, *crypticus*, is derived from the Greek stem *kryptos*, meaning hidden or secret (Brown, 1956) and is in reference to this species' secretive habits, and for having kept its identity in secret for so long. The common name refers to Little Chucky Creek, the location of the only known extant population of this species.

## DISCUSSION

Taxonomic interpretations of variation in any character set are problematic but are particularly difficult when character variation is subtle. Taylor's (1969) revision of *Noturus* demonstrated that external morphological characters that are generally informative of the taxonomic status of fish populations are conserved among madtoms. The paucity of species descriptions since Taylor's (1969) revision attests in part to the difficulty of interpreting subtle variation among madtom populations, particularly in aspects of pigmentation.

However subtle, morphological variation among populations attributed to Noturus elegans is partitioned into three distinct units (Table 4), corresponding to the Green, Duck, and Nolichucky river populations. Modal differences in anal- and pelvic-fin rays, shape as assessed by multivariate analysis, and pigmentation distinguish the three populations morphologically. Also, each population is monophyletic for Cytb, and sequence divergence among populations averages 6.4%. Further, populations of N. elegans are characterized by unique allozyme phenotypes and presumptive genotypes, differing on average by fixed or nonoverlapping genotype distributions for eight of 30 allozyme loci. Partitions in allozymic, morphological, and mtDNA variation among populations assigned to N. elegans are concordant and support recognition of two new species, N. crypticus and N. fasciatus. Following Johnson et al. (2004), N. crypticus and N. fasciatus are phenotypic and phylogenetic species. Ecological attributes have not been examined, and the populations are allopatric, precluding tests of N. crypticus and N. fasciatus as ecological and biological species.

The addition of two species here brings the total number of madtom catfishes to 28, the most speciose group of catfishes in North America. However, the degree of morphological versus genetic divergence between Noturus crypticus and N. fasciatus is consistent with the suggestions of morphological conservatism and considerable undetected taxonomic diversity in this component of the North American ichthyofauna (Burr and Stoeckel, 1999). Evidence of cryptic speciation in other elements of the madtom fauna includes N. albater, which Taylor (1969) described from Ozark drainages of Missouri and Arkansas. Taylor (1969) reported no appreciable variation in meristic and morphometric characters or pigmentation, but Grady (1987) and Grady and LeGrande (1992) reported strongly partitioned allozymic and chromosomal variation that supported recognition of eastern and western Ozark species.

A surprising number of madtom species, including *Noturus stanauli* (Etnier and Jenkins, 1980) described since Taylor (1969), are both narrowly distributed and extremely rare, key indicators of extinction risk. Not surprisingly, many of these apparently relict species are recognized as threatened or endangered (U.S. Fish and Wildlife Service, 1994). Already, one madtom species, *N. trautmani*, is presumed extinct, having last been collected in 1957.

As presently understood, *Noturus crypticus* also is rare and geographically limited, being restricted to Little Chucky Creek, Greene Co., Tennessee, in the Ridge and Valley Province of the Nolichucky River system, Tennessee River drainage. Comprehensive studies of madtom catfishes, including taxonomic assessments and analyses of the origin and distribution of species, will yield insights that could help preserve this component of the North American ichthyofauna and, more generally, on the characteristics and history of species that contribute to extinction risk.

## MATERIAL EXAMINED

Numbers in parentheses are numbers of specimens measured followed by the number used for meristic data.

Noturus elegans.—Kentucky: USNM 217383 (0, 12), Adair Co., Green R., at KY 70 crossing, just E of Liberty, 11 Sept. 1976. SIUC 11601 (10, 0), Adair Co., Green R., 200 m upstream from KY76 crossing, 22 Sept. 1982. SIUC 0029 (2, 4), Allen Co., Long Cr., 1.6 km SW Amos, 29 Oct. 1980. SIUC 11489 (7, 10), Allen Co., Long Cr., 1.6 km E KY 100-KY 67 junction in Oak Grove, 15 July 1982. SIUC 0048 (6, 10), Allen Co., Trammel Cr., 3.2 km SW of Halfway at route 1332 bridge, 25 May 1981. SIUC 11104 (7, 9), Allen Co., Trammel Fork, at old state road ford, 1.55 km N of Red Hill, 14 July 1982. SIUC 3834 (0, 10), Barren Co., Fallen Timber Cr., 1.6 km NW Eighty-eight, 26 Sept. 1981. SIUC 10536 (0, 6), Casey Co., Green R., at Rupert Ford, 1.5 km NNW of Antioch Church, 22 Sept. 1982. SIUC 11425 (0, 4), Green Co., Russell Cr., 0.6 km S KY 487 at Branlett, 23 Sept. 1982. SIUC 10236 (3, 0), Green Co., Green R., at Greensburg, 21 July 1984. SIUC 1086 (0, 11), same data as preceding, 21 June 1980. SIUC 10218 (0, 11), Green Co., Green R., at Roachville crossing, 21 July 1984. SIUC 11645 (2,0), Monroe Co., East Fork Barren R., at mouth of Isenburg Cr., 12 June 1979. SIUC 1795 (2, 2), Monroe Co., Indian Cr., 4.8 km E Fountain Run, 25 May 1981. SIUC 1808 (0, 1), same data as preceding, 18 March 1980. SIUC

11467 (0, 2), Monroe Co., Barren R., at ford on Deep Ford Road, 1.25 km SSE Old Walnut Grove School, 15 July 1982. SIUC 7742 (0, 2), Monroe Co., East Fork Barren R., at KY 100 bridge and KY 792, 12 June 1979. SIUC 10793 (0, 1), Taylor Co., Pitman Cr., at its intersection with Chaney Pike Road, 2.2 km ESE White Rose, 27 Aug. 1983. SIUC 10781 (0, 1), Taylor Co., Stoner Cr., directly above its junction with Old School Road, 5.3 km E Acton, 26 Oct. 1982. SIUC 10774 (0, 1), Taylor Co., Locust Lick Br., 1.4 km NE Bengal, 19 Aug. 1983. SIUC 11001 (0, 1), Warren Co., Gasper R., at KY 626 crossing, 3.25 km SSW KY 231-KY 626 junction, 9 July 1982.

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## LITERATURE CITED

AVISE, J. C., AND R. M. BALL. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxford Surv. Evol. Biol. 7:45–67.

- BOOKSTEIN, F. L., B. CHERNOFF, R. L. ELDER, J. M. HUMPHRIES, JR., G. R. SMITH, AND R. E. STRAUSS. 1985. Morphometrics in evolutionary biology. Acad. Nat. Sci., Phil. Spec. Publ. 15:1–277.
- BOSCHUNG, H. T., AND R. L. MAYDEN. 2004. Fishes of Alabama. Smithsonian Institution Press, Washington, D.C.
- BROWN, R. W. 1956. Composition of Scientific Words. Smithsonian Institution Press, Washington, D.C.
- BURR, B. M., AND W. W. DIMMICK. 1981. Nests, eggs and larvae of the Elegant Madtom, *Noturus elegans* from Barren River drainage, Kentucky (Pisces:Ictaluridae). Trans. Kentucky Acad. Sci. 42:116–118.
- ——, AND J. N. STOECKEL. 1999. The natural history of madtoms (genus *Noturus*), North America's diminutive catfishes, p. 51–101. *In:* Catfish 2000: Proceedings of the International Ictalurid Symposium. E. R. Irwin, W. A. Hubert, C. F. Rabeni, H. L. Schramm, Jr, and T. Coon (eds.). Amer. Fish. Soc. Symp. 24, Bethesda, Maryland.
- ——, AND M. L. WARREN, JR. 1986. A distributional atlas of Kentucky fishes. Kentucky State Nat. Preserves Comm. Sci. Tech. Series 4:1–398.
- CASHNER, R. C., J. S. ROGERS, AND J. M. GRADY. 1988. *Fundulus bifax*, a new species of the subgenus *Xenisma* from the Tallapoosa and Coosa river systems of Alabama and Georgia. Copeia 1988:674– 685.
- CICERELLO, R. R., AND G. A. SCHUSTER. 2003. A guide to the freshwater mussels of Kentucky. Kentucky State Nat. Preserves Comm. Sci. Tech. Series 7:1–62.
- CLAY, W. M. 1975. The Fishes of Kentucky. Kentucky Dept. Fish Wild. Res., Frankfort, Kentucky.
- DINKINS, G. R., AND P. W. SHUTE. 1996. Life histories of *Noturus baileyi* and *N. flavipinnis* (Pisces: Ictaluridae), two rare madtom catfishes in Citico Creek, Monroe County, Tennessee. Bull. Alabama Mus. Nat. Hist. 18:43–69.
- DOUGLAS, N. H. 1972. Noturus taylori, a new species of madtom (Pisces, Ictaluridae) from the Caddo River, southwest Arkansas. Copeia 1972:785–789.
- EISENHOUR, D. J., B. M. BURR, K. M. COOK, AND C. A. TAYLOR. 1996. Conservation status review of the Saddled Madtom, *Noturus (Rabida)* sp. (Siluriformes: Ictaluridae) in the Duck River system, Tennessee. J. Tenn. Acad. Sci. 71:41–46.
- ETNIER, D. A. 1997. Jeopardized southeastern freshwater fishes: a search for causes, p. 87–104. *In:* Aquatic Fauna in Peril: The Southeastern Perspective. G. W. Benz and D. W. Collins (eds.). Southeast Aquatic Res. Inst. Special Pub. 1, Lenz Design & Communications, Decatur, Georgia.
- ——, AND R. E. JENKINS. 1980. *Noturus stanauli*, a new madtom catfish (Ictaluridae) from the Clinch and Duck rivers, Tennessee. Bull. Alabama Mus. Nat. Hist. 5:17–22.
- —, AND W. C. STARNES. 1991. An analysis of Tennessee's jeopardized fish taxa. J. Tennessee Acad. Sci. 66:129–133.
- —, AND —, 1993. The Fishes of Tennessee. Univ. Tennessee Press, Knoxville, Tennessee.

- FEEMAN, J. C., JR. 1987. Results of fish surveys in the Tennessee River drainage, 1979–1981. Brimleyana 13:99–121.
- GRADY, J. M. 1987. Biochemical systematics and evolution of the ictalurid catfish genus *Noturus* (Pisces, Siluriformes). Unpubl. Ph.D. Diss., Southern Illinois Univ., Carbondale, Illinois.
- ——, AND W. H. LEGRANDE. 1992. Phylogenetic relationships, modes of speciation, and historical biogeography of the madtom catfishes, genus *Noturus* Rafinesque (Siluriformes: Ictaluridae), p. 747–777. *In:* Systematics, Historical Ecology, and North American Freshwater Fishes. R. L. Mayden (ed.). Stanford Univ. Press, Stanford, California.
- HARDMAN, M. 2004. The phylogenetic relationships among *Noturus* catfishes (Siluriformes: Ictaluridae) as inferred from mitochondrial gene cytochrome *b* and nuclear recombination activating gene 2. Mol. Phylogenet. Evol. 30:395–408.
- HUBBS, C. L., AND K. F. LAGLER. 1974. Fishes of the Great Lakes Region. Univ. Michigan Press, Ann Arbor, Michigan.
- HUMPHRIES, J. M., F. L. BOOKSTEIN, B. CHERNOFF, G. R. SMITH, R. L. ELDER, AND S. G. POSS. 1981. Multivariate discrimination by shape in relation to size. Syst. Zool. 30:291–308.
- JOHNSON, J., T. DOWLING, AND M. BELK. 2004. Neglected taxonomy of rare desert fishes: congruent evidence for two species of leatherside chub. Syst. Biol. 53:841–855.
- LEGRANDE, W. H. 1981. Chromosomal evolution in North American catfishes (Siluriformes: Ictaluridae), with particular emphasis on the madtoms, *Noturus*. Copeia 1981:33–52.
- MAYDEN, R. L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem, p. 381–424. *In:* The Units of Biodiversity.M. F. Claridge, H. A. Dawah, and M. R. Wilson (eds.). Chapman and Hall, London.
- ——, AND B. KUHAJDA. 1989. Systematics of *Notropis* cahabae, a new cyprinid fish endemic to the Cahaba River of the Mobile Basin. Bull. Alabama Mus. Nat. Hist. 9:1–16.
- METTEE, M. F., P. E. O'NEIL, AND J. M. PIERSON. 1996. Fishes of Alabama and the Mobile Basin. Oxmoor House, Birmingham, Alabama.
- MURPHY, R. W., J. W. SITES, JR., D. G. BUTH, AND C. H. HAUFLER. 1996. Chapter 4. Proteins: isozyme electrophoresis, p. 51–120. *In:* Molecular Systematics, 2nd ed. D. M. Hillis, C. Moritz, and B. K. Mable (eds.). Sinauer Associates, Sunderland, Massachusetts.
- ORTH, D. J. 1983. Aquatic habitat measurements, p. 61–84. *In:* Fisheries Techniques. L. A. Nielsen and D. L. Johnson (eds.). American Fisheries Society, Bethesda, Maryland.
- PAGE, L. M., AND B. M. BURR. 1991. A Field Guide to Freshwater Fishes, North America North of Mexico. Houghton Mifflin Co., Boston, Massachusetts.
- PALUMBI, S. R. 1996. Chapter 7. Nucleic acids II: the polymerase chain reaction, p. 205–247. *In:* Molecular Systematics, 2nd ed. D. M. Hillis, C. Moritz,

and B. K. Mable (eds.). Sinauer Associates, Sunderland, Massachusetts.

- Poss, S. G., AND B. B. COLLETTE. 1995. Second survey of fish collections in the United States and Canada. Copeia 1995:48–70.
- RAMSEY, J. S., H. T. BOSCHUNG, W. M. HOWELL, T. S. JANDEBEUR, M. F. METTEE, R. D. NESTER, P. E. O'NEILL, M. PIERSON, W. C. REEVES, R. A. STILES, AND W. WIELAND. 1984. Freshwater fishes, p. 1–14. *In:* Vertebrate Wildlife of Alabama. R. H. Mount (ed.). Alabama Agricultural Experiment Station, Auburn Univ., Auburn, Alabama.
- ROHDE, F. C. 1980. Noturus elegans Taylor, Elegant Madtom, p. 450. In: Atlas of North American Freshwater Fishes. D. S. Lee, C. R. Gilbert, C. H. Hocutt, R. E. Jenkins, D. E. McAllister, and J. R. Stauffer, Jr (eds.). North Carolina State Museum of Natural History, Raleigh, North Carolina.
- STARNES, W. C., AND D. A. ETNIER. 1986. Drainage evolution and fish biogeography of the Tennessee and Cumberland Rivers drainage realm, p. 325– 361. *In:* The Zoogeography of North American Freshwater Fishes. C. H. Hocutt and E. O. Wiley (eds.). John Wiley and Sons, New York.
- STEIN, D. W., J. S. ROGERS, AND R. C. CASHNER. 1985. Biochemical systematics of the *Notropis roseipinnis* complex (Cyprinidae: subgenus *Lythrurus*). Copeia 1985:154–165.
- STRAUSS, R. E., AND F. L. BOOKSTEIN. 1982. The truss: body form reconstruction in morphometrics. Syst. Zool. 31:113–135.
- TAYLOR, W. R. 1969. A revision of the catfish genus *Noturus* Rafinesque with an analysis of higher groups in the Ictaluridae. Bull. U.S. Nat. Mus. 282:1–315.
- THOMAS, M. A., AND B. M. BURR. 2004. Noturus gladiator, a new species of madtom (Siluriformes: Ictaluridae) from Coastal Plain streams of Tennessee and Mississippi. Ichthyol. Explor. Freshwaters 15:351–368.
- TRAUTMAN, M. B. 1981. The Fishes of Ohio, revised edition. Ohio State Univ. Press, Columbus, Ohio.
- U.S. FISH AND WILDLIFE SERVICE. 1994, Endangered and threatened wildlife and plants 50 CFR 17.11 and 17.12 July 15, 1994. Compiled from rulings in the Federal Register. U.S. Fish and Wildlife Service, Publication Unit, Washington, D.C.

- WARREN, M. L., JR., P. L. ANGERMEIER, B. M. BURR, AND W. R. HAAG. 1997. Decline of a diverse fish fauna: patterns of imperilment and protection in the southeastern United States, p. 105–164. *In:* Aquatic Fauna in Peril: The Southeastern Perspective. G. W. Benz and D. W. Collins (eds.). Southeast Aquatic Research Inst. Special Publ. 1, Lenz Design & Communications, Decatur, Georgia.
- ——, B. M. BURR, AND J. M. GRADY. 1994. *Notropis albizonatus*, a new cyprinid fish endemic to the Tennessee and Cumberland river drainages, with a phylogeny of the *Notropis procne* species group. Copeia 1994:868–886.
- —, —, S. J. WALSH, H. L. BART, JR., R. C. CASHNER, D. A. ETNIER, B. J. FREEMAN, B. R. KUHAJDA, R. L. MAYDEN, H. W. ROBISON, S. T. ROSS, AND W. C. STARNES. 2000. Diversity, distribution, and conservation status of the native freshwater fishes of the southern United States. Fisheries 25:7–29.
- WIENS, J. J., AND T. A. PENKROT. 2002. Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). Syst. Biol. 51:69–91.
- WILCOX, T. P., F. J. GARCIA DE LEON, D. A. HENDRICK-SON, AND D. M. HILLIS. 2004. Convergence among cave catfishes: long-branch attraction and a Bayesian relative rates test. Mol. Phylogenet. Evol. 31:1101–1113.
- WILLIAMS, J. D., M. L. WARREN, JR., K. S. CUMMINGS, J. L. HARRIS, AND R. J. NEVES. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18:6–22.
- (BMB) DEPARTMENT OF ZOOLOGY, SOUTHERN ILLINOIS UNIVERSITY, CARBONDALE, ILLINOIS 62901; (DJE) DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES, MOREHEAD STATE UNIVERSITY, MOREHEAD, KENTUCKY 40351; AND (JMG) DEPARTMENT OF BIOLOGICAL SCIENCES, UNIVERSITY OF NEW ORLEANS, NEW ORLEANS, LOUISIANA 70122. E-mail: (BMB) burr@ zoology.siu.edu. Send reprint requests to BMB. Submitted: 13 Dec. 2004. Accepted: 15 Aug. 2005. Section editor: J. W. Armbruster.

Enzyme (Enzyme Commission number)	Locus	Tissue source
Aconitate hydratase (4.2.1.3)	ACOH-A	muscle
Adenylate kinase (EC 2.7.4.3)	AK-A	eye, muscle
Alcohol dehydrogenase (1.1.1.1)	ADH-A	liver
Aspartate aminotransferase (2.6.1.1)	mAAT-A	liver, muscle
•	sAAT-A	liver, muscle
Calcium binding protein	CBP	liver, muscle
Creatine kinase (2.7.3.2)	CK-A	eye, muscle
	CK-B	muscle
Cytosol aminopeptidase (3.4.11.1)	CAP-A	liver, muscle
Fumarate hydratase (4.2.1.2)	FUMH-A	muscle
Glucose-6-phosphate dehydrogenase (1.1.1.118)	G6PDH-A	liver
Glucose-6-phosphate isomerase (5.3.1.9)	GPI-A	liver, muscle
· ·	GPI-B	muscle
Glycerol-3-phosphate dehydrogenase (1.1.1.8)	G3PDH-A	muscle
Isocitrate dehydrogenase (1.1.1.42)	mICDH-A	muscle
	sICDH-A	liver
Lactate dehydrogenase (1.1.1.27)	LDH-A	muscle
	LDH-B	liver, muscle
Malate dehydrogenase (1.1.1.37)	mMDH-A	muscle
	sMDH-A	liver, muscle
	sMDH-B	muscle
Malate dehydrogenase (1.1.1.40)	MDHP-A	liver
Mannose-6-phosphate isomerase (5.3.1.8)	MPI-A	muscle
Peptidases (3.4.13.9)	Pep-A	muscle
	Pep-B	muscle
	Pep-D	muscle
Phosphoglucomutase (5.4.2.2)	PGM-A	muscle
Phosphogluconate dehydrogenase (1.1.1.44)	PGDH-A	liver, muscle
Pyruvate kinase (2.7.1.40)	PK-A	muscle
Superoxide dismutase (1.15.1.1)	SOD-A	liver

APPENDIX 1. ENZYME SYSTEMS, PRESUMPTIVE LOCI EXAMINED, AND TISSUE SOURCES. Enzyme nomenclature follows Murphy et al. (1996).