

NOTE

REDISCOVERY OF THE HEADWATER CATFISH *ICTALURUS LUPUS*
(ICTALURIDAE) IN A WESTERN GULF-SLOPE DRAINAGE

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ABSTRACT—Stream flow modifications, such as reduced spring flow and construction of low-head dams, have led to declines in the headwater catfish (*Ictalurus lupus*) across much of its range, as has competition with channel catfish (*I. punctatus*). Hybridization between headwater catfish and channel catfish also poses a threat to the headwater catfish. Our analyses of cytochrome-*b* sequences of headwater catfish from the Frio River, Devils River, and Independence Creek indicated that a population of headwater catfish occurs in the Frio River of the Nueces Drainage, where it was considered extirpated since 1967, and that hybridization is occurring in populations that inhabit the Frio River and Independence Creek. No obvious sign of hybridization was present in the population of headwater catfish in the Devils River.

RESUMEN—La modificación de flujo de arroyos como reducida alimentación por manantiales y la construcción a pequeña escala de diques ha provocado la disminución del bagre lobo (*Ictalurus lupus*) a lo largo de mucha de su distribución geográfica, así como la competencia con el bagre de canal (*I. punctatus*). Hibridación entre el bagre lobo y el bagre de canal también amenaza al bagre lobo. Nuestro análisis de secuencias de citocromo-*b* del bagre lobo de los ríos Frío y Devils y del arroyo Independence demostraron que una población del bagre lobo habita el río Frío donde fue considerada extirpada desde 1967, y que la hibridación está ocurriendo en las poblaciones del río Frío y del arroyo Independence. No hubo ninguna indicación de la hibridación en la población del bagre lobo del río Devils.

The headwater catfish *Ictalurus lupus* is a medium-sized member of the channel catfish complex (maximum total length = 431 mm; Sublette et al., 1990) that occurs in riffles, runs, and pools of spring-fed streams (Thomas et al., 2007) and is most abundant in deep, run habitats (Bonner et al., 2005). Historical distribution of the headwater catfish includes the Colorado, Guadalupe, and Nueces drainages in Texas, the Rio Grande drainage in Texas, New Mexico, and Mexico, as well as the Río San Fernando, Río Soto la Marina, Río Conchos, and the endorheic Cuatro Ciénegas Basin in Mexico (Conner and Suttkus, 1986; Kelsch and Hendricks, 1986; Edwards et al., 2003). Ranges of the headwater catfish and channel catfish *Ictalurus*

punctatus historically overlapped in the Colorado, Guadalupe, and Nueces drainages (Kelsch and Hendricks, 1990; McClure-Baker et al., 2010) where the headwater catfish occurred primarily in heavily spring-influenced streams and channel catfish occurred primarily in larger streams and rivers. However, the headwater catfish presently is considered to be extirpated from western gulf-slope drainages north of the Rio Grande (i.e., Colorado, Guadalupe, and Nueces drainages), presumably as a result of degradation of habitat, as well as competition and hybridization with the channel catfish (Kelsch and Hendricks, 1990; McClure-Baker et al., 2010). Because of declines throughout much of its range, the headwater catfish is considered to be

threatened by Jelks et al. (2008), a species of special concern by C. Hubbs et al. (http://www.utexas.edu/tmm/tnhc/fish/hubbs/HIS/Hubbs_et_al_2008_checklist.pdf) and Texas Parks and Wildlife Department (2005), and critically imperiled by the New Mexico Department of Game and Fish (Propst, 1999).

We report herein a recent collection of the headwater catfish from the Frio River in the Nueces drainage of southern Texas. This represents the first collection of the headwater catfish in the Frio River and the rediscovery of the species in the Nueces drainage, where it was last collected in 1967 (Kelsch and Hendricks, 1990).

The Frio River arises in Real County and flows southeastward through Uvalde, Medina, Frio, La Salle, McMullen, and Live Oak counties before its confluence with the Nueces River. Flows in the Frio River are primarily from groundwater discharge from the Edwards-Trinity Aquifer. Dominant substrates in the upper Frio River are cobble and gravel derived from the Cretaceous limestone that forms the Edwards-Trinity Aquifer.

Ictalurid catfishes, identified in the field as the headwater catfish, were collected and preserved as vouchers on 30 June 2007 from the Frio River at the Ranch Road 1120 (Real County, Texas) and Ranch to Market Road 1050 (Uvalde County, Texas) crossings near the city of Leakey. For comparative purposes in genetic analyses, we also collected the headwater catfish from the Devils River and Dolan Creek (at the confluence with the Devils River) above Dolan Falls (Val Verde County, Texas; $n = 9$) and Independence Creek (Terrell County, Texas; $n = 5$), and the channel catfish from the Rio Grande (Brewster County, Texas; $n = 3$), Pedernales (Gillespie County, Texas; $n = 2$), Concho (Concho County, Texas; $n = 1$), and Brazos rivers (Waller County, Texas; $n = 2$). A subset was retained as vouchers from the Devils River ($n = 2$), Independence Creek ($n = 1$), and Brazos River ($n = 2$). All vouchers were deposited in the Texas Cooperative Wildlife Collection at Texas A&M University (TCWC 13605–13609). Each individual was measured for total length (mm) and field identifications were based on counts of anal fin rays (C. Hubbs et al., http://www.utexas.edu/tmm/tnhc/fish/hubbs/HIS/Hubbs_et_al_2008_checklist.pdf) and coloration pattern. The headwater catfish has many small black spots, whereas the channel catfish has a few large black spots or no spots (Thomas et al., 2007). A sample of tissue from fins of each

specimen was preserved in 70% ethanol and subsequently stored at -80°C in the Michael R. J. Forstner Frozen Tissue Collection at Texas State University-San Marcos.

DNA was isolated using a DNeasy Tissue Kit (QIAGEN, Inc., Valencia, California). Amplification of a 1,000-bp fragment of the cytochrome-*b* mitochondrial gene was performed using primers MT11 and MT29 (Waldbieser et al., 2003) in reactions with 0.3 M TRIS, 0.075 M $(\text{NH}_4)_2\text{SO}_4$, 0.0175 M MgCl_2 , 0.1 mM dNTPs, 0.01 μM each primer, 2.5 U *Taq* polymerase, and pH = 8.5. PCR was performed in the GeneAmp PCR System 9700 (Perkin Elmer, Boston, Massachusetts) for 35 cycles, each consisting of denaturing at 95°C for 30 s, annealing at 50°C for 1 min, and extension at 72°C for 1 min, and with a final extension period of 72°C for 5 min. PCR products were purified with AMPure PCR Purification System (Agencourt Bioscience Corporation, Beverly, Massachusetts) and cycle sequenced with the above primers, using a CEQ DTCS Quick Start Kit (Beckman Coulter, Inc., Fullerton, California). Thermal cycling was 30 cycles of 96°C for 20 s, 55°C for 20 s, and 60°C for 4 min. Three internal primers were used to achieve bidirectional sequencing: MT30, MT31 (Waldbieser et al., 2003), and MT30DJM (5'-GCA ACA CTA ACC CGA TTC TTT-3'). Products were cleaned with CleanSEQ Dye Terminator Removal (Agencourt Bioscience Corporation, Beverly, Massachusetts) and analyzed on a CEQ 8800 Genetic Analysis System (Beckman Coulter, Inc., Fullerton, California). Sequences were edited and aligned in Sequencher Version 4.5 (Gene Codes Corporation, Ann Arbor, Michigan). The flathead catfish *Pylodictis olivaris* was used as an outgroup. All sequences were deposited at GenBank (accessions GQ396767–GQ396797).

Maximum-parsimony analysis was conducted using PAUP* (Swofford, 2002). The most-parsimonious tree was found using a full-heuristic search with simple stepwise addition and tree bisection-reconnection. A non-parametric bootstrap analysis was implemented for 1,000 replications with 10 random stepwise-addition sequences each, and the resulting 50% consensus topology was retained. Additional model-based phylogenetic evaluations under alternative reconstruction criteria also were conducted.

Voucher specimens were further identified using the canonical-discriminant analysis of

Kelsch (1995) that takes into account multiple morphological and meristic characteristics including length of pectoral spine, width of mouth, depth of caudal peduncle, and number of anal fin rays. Canonical scores from this analysis typically range from -4 to 4 with negative scores indicating the headwater catfish and positive scores indicating the channel catfish (Kelsch, 1995). Canonical scores near zero should indicate possible hybrid individuals with characteristics intermediate between parental species, whereas a strong score and conflicting mitochondrial haplotype indicates hybridization and potential introgression. Two voucher specimens of the channel catfish from the Brazos River also were examined to evaluate strengths of canonical scores of specimens entirely outside of the historical range of the headwater catfish.

Canonical scores of four voucher specimens from the Frio River were consistent with identification as the headwater catfish in the field (Fig. 1). One specimen had an intermediate score of 0.0006 that was not congruent with the initial identification as a headwater catfish. However, this specimen did possess a coloration pattern characteristic of the headwater catfish. Canonical scores of vouchers from the Brazos River, Devils River, and Independence Creek were consistent with identifications made in the field.

All phylogenetic analyses of the cytochrome-*b* dataset resolved two markedly divergent clades, hence only parsimony results are depicted (Fig. 1) with 100% bootstrap support for the node separating the clades. One clade (*I. lupus* lineage) was associated only with vouchers identified as the headwater catfish on the basis of morphology. The other clade (*I. punctatus* lineage) included all voucher specimens of the channel catfish and eight fish identified as the headwater catfish either in the field or both in the field and on the basis of the canonical-discriminant function. Sequence divergence between these two clades was 7.4–7.8%.

The 14 individuals in the *I. lupus* lineage were identical in sequence and the single haplotype occurred in both the Frio and Devils rivers. McClure-Baker et al. (2010) detected only two haplotypes of the headwater catfish, one in the Pecos drainage and one in the Rio Grande drainage below the Pecos River. Yates et al. (1984) and Kelsch and Hendricks (1986) also reported low levels of polymorphism for allo-

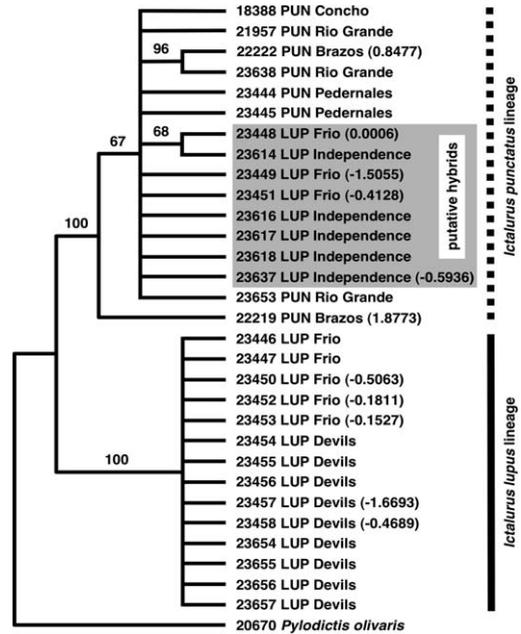


FIG. 1—Maximum-parsimony cladogram of 31 individuals (11 cytochrome-*b* haplotypes) rooted with the flathead catfish *Pyloodictis olivaris*. Bootstrap values are shown above branches. The dashed vertical bar indicates the channel catfish *Ictalurus punctatus* lineage and the black vertical bar indicates the headwater catfish *I. lupus* lineage. Individuals are represented by a unique number followed by a designation of species (LUP or PUN), which reflects identification in the field based on external morphological characters and locality. Numbers in parentheses are canonical scores for morphological identification following Kelsch (1995). Putative hybrids that were identified in the field as *I. lupus* but had a haplotype of *I. punctatus* are highlighted in gray.

zyme markers across much of the range of the headwater catfish, suggesting that genetic diversity has been low historically.

Previously, the headwater catfish was considered to have been extirpated by 1972 from the western gulf-slope drainages north of the Rio Grande (Kelsch and Hendricks, 1990). Our collections from the Frio River represent the first headwater catfish from these drainages since then, and the first collection from the Nueces-Frio drainage since 1967 (Kelsch and Hendricks, 1990). The discovery of the population in the Frio River provides an additional management unit for conservation of the species.

Haplotypes of both the headwater catfish and channel catfish were present in the population sampled from the Frio River. Two individuals had haplotypes incongruent with morphological identifications, indicating that hybridization is occurring between the headwater catfish and channel catfish in the Frio River. In our study, hybridization also was detected in Independence Creek, but was not detected in the Devils River. McClure-Baker et al. (2010) also reported high rates of hybridization in Independence Creek and detected only the haplotype of the headwater catfish in the Devils River. However, several of their specimens from the Devils River were morphologically similar to *I. punctatus*, indicating that introgression has occurred. Yates et al. (1984) also detected backcrossing of hybrids with the headwater catfish, but suggested that significant introgression between the headwater catfish and channel catfish was not occurring. The occurrence of catfish in Frio River and Independence Creek with canonical scores indicative of the headwater catfish but haplotypes identifying them as the channel catfish indicates that hybridization has occurred. Thus, hybridization and possible introgression might be of significant conservation concern for the headwater catfish as a potential mechanism by which declines of the headwater catfish continue to occur.

Further collections in the Guadalupe and Colorado river drainages should be made to re-evaluate current distribution of the headwater catfish. Additionally, because the channel catfish likely now occurs throughout the range of the headwater catfish due to introductions associated with recreational fisheries and aquaculture, a rangewide survey using bi-parentally inherited markers should be undertaken. This would aid in establishing conservation priorities by evaluating suspicions of ongoing and significant introgression and by serving to identify genetically pure populations of the headwater catfish.

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