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MOLECULAR SYSTEMATICS AND SPECIATION OF THE GILT DARTER (*Percina evides*) IN THE ST. CROIX RIVER DRAINAGE.

FINAL REPORT

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ABSTRACT

Systematics and evolutionary history of the widespread gilt darter (*Percina evides*) was investigated with complete sequences of the mitochondrial encoded cytochrome *b* gene. Most disjunct populations throughout the gilt darter's range were sampled to assess the taxonomic status of populations in the upper Mississippi River of Minnesota and Wisconsin. Two major lineages were recovered in phylogenetic analysis, a western clade containing upper Mississippi River and Ozark populations, and a clade containing all populations east of the Mississippi River. The phylogeography of *P. evides* is discussed and the populations in Minnesota are hypothesized to have resulted from post-Pleistocene dispersal from the Ozarks. The upper Mississippi populations are not morphologically distinct from the Ozark populations; however, these populations are morphologically and genetically divergent from eastern populations and represent an undescribed species. The conservation implications of phylogeography of *P. evides* is discussed.

INTRODUCTION:

The gilt darter, *Percina evides*, has a wide range throughout the Mississippi River Basin from New York to Minnesota and south to northern Alabama and northern Arkansas (Page, 1983). Based on collection records, the range of *P. evides* was probably never continuous in historical times, and populations that have been extirpated from Illinois, Iowa, and Ohio (Denoncourt, 1969; Smith, 1979; Trautman, 1981) may have been small and headed for extinction at the time of European settlement. Currently *P. evides* is distributed as highly disjunct populations in the Ozarks of Missouri and Arkansas, the Eastern Highlands of Tennessee, Kentucky, and North Carolina, the Allegheny River in Pennsylvania and New York, the Wabash Drainage in Indiana, and the St. Croix, Black, and Chippewa rivers of Minnesota and Wisconsin (Becker, 1983; Page, 1983).

An unpublished Ph.D. thesis (Denoncourt, 1969) studied morphological variation across the entire range of *P. evides* and revealed significant variation among the disjunct populations. The populations in the Ozarks and Upper Tennessee River Drainage were each recognized as distinct, undescribed subspecies. All other populations, including the St. Croix Drainage, were grouped into the nominate subspecies, *P. e. evides*. However, the data presented in Denoncourt (1969) clearly demonstrate that the St. Croix Drainage population is statistically different from other populations of *P. e. evides* from the Ohio, Wabash, Tennessee, and Cumberland drainages for a

number of systematically informative characters. The populations that are most similar morphologically to the St. Croix R. *P. evides* appear to be those from the Ozarks (Denoncourt, 1969); even though these areas are separated by over 800 km. The systematic affinities of *P. evides* populations that existed in Iowa and Illinois have been difficult to assess because only two specimens, collected in the 1880s, are present in museum collections (Denoncourt, 1969).

Three hypotheses invoking dispersal or vicariance have been forwarded to explain the presence of *P. evides* in the St. Croix, Black, and Chippewa rivers. All three of these hypotheses differ in the estimate of the timing of isolation and the age of the upper Mississippi River populations. Post-Pleistocene dispersal from an unidentified southern population to the upper Mississippi River was proposed by Denoncourt (1969). Alternatively, Pflieger (1971) hypothesized that *P. evides* may have been isolated in the Driftless Area, Ozarks, and Eastern Highlands during the Wisconsin glaciation. Presumably, this would involve dispersal from a southern population of *P. evides* to the Driftless Area during the Sangamonian Interglacial Period (approximately 50,000 years ago [Frey et al., 1965]). The third hypothesis proposes that *P. evides* was widely distributed throughout the ancestral Teays River System, prior to the obliteration of its northern and western channels in the Nebraskan and Kansan periods of the Quaternary (Mayden, 1988). According to this hypothesis, populations of *P. evides* in the upper Mississippi

are ancient relicts and have persisted in Driftless Area glacial refugia at least since the early periods of the Quaternary. A number of other freshwater fishes exhibit disjunct distributions in the upper Mississippi and these hypotheses have also been used to explain the distributions of these species (Pflieger, 1971; Mayden, 1988). These include *Campostoma oligolepis* (largescale stoneroller), *Notropis nubilis* (Ozark minnow), and *Etheostoma zonale* (banded darter) (Pflieger, 1971).

All three of these hypotheses have specific predictions pertaining to the phylogenetic and population genetic structure within the upper Mississippi River populations. The hypotheses of Quaternary and post-Quaternary dispersal to this area from a southern population would be supported if the upper Mississippi river populations were phylogenetically nested within the southern source population. The origin of the upper Mississippi populations in the late Quaternary (Pflieger, 1971) or the post-Pleistocene is too recent to expect the accumulation of significant genetic differences in mitochondrial genes between from the southern source population(s). The upper Mississippi river populations should be genetically homogenous with the source populations and are not expected to be reciprocally monophyletic.

The vicariance hypothesis of Mayden (1988) predicts that populations of *P. evides* currently distributed in the former Teays River (Licking, Kentucky, Elk, and Allegheny), upper Mississippi, and Ozarks should form a monophyletic group. Populations in the Old Ohio River (Wabash and Green),

Cumberland, and Tennessee drainages should form a second monophyletic group within *P. evides*. This phylogeography predicts a wide pre-Quaternary distribution of *P. evides*, which was disrupted and fragmented by the diversion of the Teays River from flowing north to the upper Mississippi to south into the Old Ohio River (Mayden, 1988). The recovery of polyphyly among the extant populations in the Ohio Drainage would support the hypothesis that *P. evides* was extant in these rivers prior to the Nebraskan glacial advance. Monophyly of populations in the Ohio Drainage would suggest recent dispersal of *P. evides* within this drainage. If the upper Mississippi river population was isolated by the time of the Nebraskan glacial advance, then appreciable genetic differences would be expected to have accumulated. Additionally, the upper Mississippi River populations should be genetically distinct and reciprocally monophyletic within a monophyletic Teays River-Ozark clade.

The use of mitochondrial DNA sequences will permit an examination of the phylogeography of *P. evides*, providing a historical framework through a hypothesis of phylogenetic relationships among the disjunct populations. The phylogeographic analysis will permit an assessment of taxonomic status of populations and provide a test of the competing hypotheses pertaining specifically to the origin and age of the upper Mississippi River populations. The utility of mitochondrial DNA sequence data in phylogeographic analyses is well documented (Avise, 1994) and includes the ease of data collection, the

presence of nucleotide character classes that range from rapid to conservative rates of mutation, and the availability of a multitude of comparative sequences from other species of fishes.

Intraspecific analyses of cytochrome b variation have been used to study phylogeography, processes of speciation, and to assess species status in darters. Wiley and Hagen (1997) analyzed partial sequences of cytochrome b from the conservative 5' portion of the gene in sand darters (*Ammocrypta*) and demonstrated speciation patterns of *Ammocrypta beani* associated with stream evolution in the Mobile Basin. In an unpublished study of intra- and interspecific divergences and processes of speciation in the monophyletic *Percina* subgenus *Swainia*, levels of cytochrome b sequence divergence between monophyletic species range from 1.8% to 6.1%. The range of cytochrome b interspecific sequence divergence in other fish sister species with a hypothesized allopatric origin range from 2.0% to 6.3% (McCune and Lovejoy, 1998). The range of maximal intraspecific cytochrome b divergence reported ranges from 0.01% to 5.8 %; however, all species listed are not recovered as monophyletic when analyzed with closely related species (Zhu et al., 1994; McCune and Lovejoy, 1998). For monophyletic species, the range of intraspecific divergence is 0.01% to 1.5% (McCune and Lovejoy, 1998).

The utility and limits of comparative mitochondrial DNA sequence divergence to designate and diagnose taxonomic units is a subject of considerable debate, with particularly divergent opinions (Moritz, 1994;

Patton and Smith, 1994; Mayden and Wood, 1995). This issue is of prime importance for defining and designating conservation units (Moritz, 1994; Vogler and DeSalle, 1994). The concept of the Evolutionary Significant Unit (ESU) has its origin in the conservation biology literature and it is used to designate "a set of populations with a distinct, long-term evolutionary history" (Moritz, 1994). In defining the ESU, Moritz provides a qualitative requirement that ESUs should show reciprocal monophyly of mtDNA alleles and significant differences in allele frequencies at nuclear encoded loci (Moritz, 1994). The requirement of nuclear gene divergence is to ensure that populations are not connected by nuclear gene flow, which occurs in species with male-biased dispersal (Moritz, 1994).

Other definitions of ESUs rely on the phylogenetic species concept (Vogler and DeSalle, 1994), which defines species as a cluster of organisms that possesses a unique and diagnosable character or combination of characters (Nixon and Wheeler, 1990). The types of characters used to diagnose phylogenetic species, and ESUs, include genetic (DNA, allozymes, etc.), morphological, behaviorial, and ecological (Vogler and DeSalle, 1994). Therefore, reciprocal monophyly of populations for mitochondrial DNA haplotypes in combination with unique combinations of morphological traits can be used as evidence to designate these populations as ESUs, as well as phylogenetic species.

P. evides is listed as a species of special concern in Minnesota and as endangered in Wisconsin. Populations of *P. evides* in the St. Croix, Black, and Chippewa rivers are considered to be peripheral populations, "isolated in recent times," occurring on the edge of a wide-ranging species (Eddy and Underhill, 1974, p. 379). There is evidence from morphological variation (Denoncourt, 1969) that these populations of *P. evides* may represent a distinct species, which is an irreplaceable individual lineage with unique reproductive and ecological characteristics. Understanding the evolutionary history, genetic variability, and taxonomic status of rare animal populations is essential to the development of successful conservation agendas, priorities, and practices (Avise, 1994; Moritz, 1994; Smith et al., 1995). If the St. Croix population of *P. evides* is found to be a distinct species and/or an ESU, it will be the northern-most endemic of the family Percidae in the world and would potentially lead to a reevaluation of the conservation status of *P. evides* in Minnesota.

This investigation was initiated to analyze nucleotide variation among the disjunct populations of *P. evides* and to combine these findings with described morphological variation (Denoncourt, 1969) to determine if *P. evides* populations in the St. Croix, Black, and Chippewa rivers represent a distinct and unrecognized species. The phylogenetic analysis of mitochondrial DNA sequence variation will also be used to reconstruct the evolutionary relationships of the disjunct of *P. evides*, which permits a test of the

alternative hypotheses concerning the geographic origin and time of isolation and diversification of St. Croix *P. evides* populations.

MATERIALS AND METHODS:

Collection of DNA sequence data.---Fishes were collected from several localities throughout the range of *P. evides* using a minnow seine and backpack electroshocker (Table 1). Several specimens designated for molecular analysis were frozen whole in liquid nitrogen and were kept at -80°C in the laboratory for long-term storage. Genomic DNA was isolated from 0.01-0.05 g of frozen muscle tissue using standard proteinase-K digestion and phenol-chloroform extraction. Nucleic acids were precipitated with 1/10 volume of 3M sodium acetate and 2.5 volumes of absolute ethanol. Precipitated DNA extractions were resuspended in 100 ul of 1X Tris-EDTA solution. Primers GLU (GAC TTG AAG AAC CAC CGT TG) and THR (TCC GAC ATT CGG TTT ACA AG) were used for the polymerase chain reaction (PCR), which amplified the complete coding sequence of the mitochondrially encoded cytochrome b. PCR conditions included the use of 0.2 mM of each dNTP, 0.5 uM of each primer, and 2.5 mM $MgCl_2$. Approximately 100 ng of template DNA was used in each PCR. The thermal cycling profile used for successful PCR amplification was a one time 3 min. denaturation at 94°C, followed by 25 cycles of 94°C (30 sec.), 55°C (30 sec), and 72°C (1.5 min.). The 25 cycles were followed by a 5 min. extension at 72°C. Successful amplifications were confirmed by electrophoresis on 1% agarose gels in 1X TBE. The amplification products from PCR were isolated using the QiaGen QIAquick™ spin filtration system.

Cycle sequencing of isolated PCR products utilized the Perkin-Elmer BigDye™ kit, following the manufacturer's recommendations. Sequences were visualized using an ABI 377 Automated Sequencer at the University of Illinois Biotechnology Center. The PCR primers were used to sequence from the 5' and 3' end of the cytochrome b and two internal primers were used to ensure that all PCR amplifications were completely sequenced for both strands.

Data analysis.---Sequences were aligned by hand to the percid cytochrome b data set of Song et al. (1998). Pairwise genetic distances, pairwise transition - transversion ratios, and base composition values were calculated using PAUP* 4.0b1. The occurrence of multiple substitutions at a given nucleotide position (saturation) was assessed by plotting observed numbers of transitions (C↔T; A↔G) vs. observed transversions (C↔G; C↔A; T↔A; T↔G).

Phylogenetic relationships were reconstructed using both maximum parsimony and maximum likelihood analyses, which were executed using PAUP* 4.0b1. Initially, all of the *P. evides* sequences were analyzed along with a dataset which included complete cytochrome b sequences from all species of *Percina* and representative species from all percid genera (Song et al., 1998). After identifying *P. aurantiaca* as the sister species of *P. evides*, it

was included with two basal species of *Percina* (*P. roanoka* and *P.* (*Alvordius*) sp.) which were designated as outgroup species for all subsequent analyses.

Branch-and-bound tree searches were used for maximum parsimony analysis. Bootstrap (2,000 pseudoreplications) analysis was used to examine levels of relative support for inferred monophyletic groups. Maximum likelihood analysis utilized a general time reversible model of nucleotide substitution, with a correction for among-site rate variation (GTR+Γ). This model was chosen using Akaike information criterion, as implemented in the Modeltest ver. 2.0 computer program. Heuristic searches were used to find the topology with the best likelihood score using tree bisection-reconnection (TBR) with steepest descent option and 20 random addition sequences. Bootstrap analysis within the maximum likelihood optimality criteria utilized 100 pseudoreplications.

RESULTS AND DISCUSSION:

Cytochrome b variation.---Uncorrected *p*-distances from 13 individual *P. evides* from 12 populations ranged from 0.0000 to 0.0404 substitutions per site (0 to 4.04% divergent). Plotting numbers of transitions vs. transversions did not detect saturation of nucleotide substitutions (plot not shown). Two hierarchical patterns of sequence divergence are apparent (Table 2). First, divergences among the Upper Mississippi and Ozark populations are shallow (0.0009 to 0.0228 substitutions per site; mean = 0.0129), as are divergences among the Ohio and Tennessee River populations (0.0000 to 0.0061 substitutions per site; mean = 0.0003). Second, there is substantial sequence divergence when western populations and eastern populations are compared (0.0272 to 0.0404 substitutions per site; mean = 0.0320). Within the Ozarks and upper Mississippi populations there are two distinct levels of divergence. Populations within the northern Ozarks (Missouri River Drainage) and the upper Mississippi River Drainage (St. Croix, Snake, and Black rivers) exhibit a mean sequence divergence of only 0.0001 substitutions per site. The two sampled populations from the southern Ozarks (White River Drainage) exhibit 0.0053 substitutions per site. Divergences are moderate between the northern Ozark-upper Mississippi and the southern Ozark populations (mean = 0.0226 substitutions per site).

Among the 1,140 nucleotides sequenced for 13 *P. evides*, 63 sites are variable and 55 of these sites are at the third codon position. There is only

one polymorphic second codon nucleotide in one of two individuals sampled from the Tippecanoe River (Wabash-Ohio Drainage). It is an A↔G transition and results in a conservative amino acid change. There are three other amino acid substitutions which involve first codon position nucleotide changes. Three of the four polymorphic amino acids involve a change in a single individual; however, at amino acid 327 all individuals from eastern populations code for alanine (Ala) and all individuals from the western populations code for threonine (Thr). This is a non-conservative change involving a shift between a hydrophobic (Ala) and hydrophilic (Thr) amino acid.

Phylogenetic relationships of P. evides *populations*.---In both maximum parsimony (Figure 1) and maximum likelihood (Figure 2) analyses, the eastern and western populations of *P. evides* were each recovered as a monophyletic group with moderate to strong bootstrap support. Within the eastern populations, mitochondrial haplotypes are so similar that there is no phylogenetic structuring of these units. In the western populations, the moderate divergence recovered between the northern Ozark-upper Mississippi and the southern Ozark populations is reflected in the recovery of each as a monophyletic group in the phylogenetic analyses (Figures 1 and 2). However, relationships within each of these population groups is unresolved due to the lack of significant mitochondrial haplotype variation.

The lack of phylogenetic resolution (Figures 1 and 2) and the shallow genetic divergences between the northern Ozark and upper Mississippi River populations do not support the hypothesis that the populations of *P. evides* in the upper Mississippi River (St. Croix, Snake, and Black rivers) represent a distinct species. Morphologically (Denoncourt, 1969), genetically (Table 2), and phylogenetically (Figures 1 and 2) these populations are indistinguishable from the northern Ozark populations.

Reciprocal monophyly of mitochondrial DNA haplotypes is the basic criterion that is used to designate different species when using molecular data (Moritz, 1994; Vogler and DeSalle, 1994); however, comparisons of sequence divergence among recognized species can aid in assessing if these specific designations reflect significant reproductive isolation (Lovette et al., 1999). Among several sister species of darters, including *Percina*, *Etheostoma*, and *Ammocrypta*, levels of sequence divergence range from 0.0061 to 0.1150 substitutions per site (Table 3). The 3.2% mean sequence divergence between eastern and western populations of *P. evides* is within the range of recognized sister species (Table 3). The monophyly of these two units in phylogenetic analyses, the presence of morphological differences in scale counts and coloration (Denoncourt, 1969; Near and Page, unpublished data), and significant levels of sequence divergence (Table 2) support the recognition of two phylogenetic species. The type locality for *P. evides* is the White River in

Indiana (Wabash Drainage); therefore, if a new species is to be described from *P. evides*, it will come from the western populations.

The Central Highlands, which includes the Eastern Highlands (Appalachian Mountains) and the Interior Highlands (Ozark and Ouachita Mountains), contain the largest concentration of freshwater fish species in North America (Page, 1983; Mayden, 1985). One hypothesis to explain the presence of such a large number of freshwater species is geologic stability: Quaternary Period glaciers never reached these areas (Pflieger, 1971). Additionally, Mayden and Wiley (1985) proposed that the concentration of species in these areas is indicative of a widespread and diverse pre-Quaternary freshwater fish fauna. As discussed above, the pre-Quaternary fauna hypothesis has been used to address the origin of *P. evides* in the upper Mississippi River (Mayden, 1988).

Several sister species or clades of freshwater fishes are found respectively in the Eastern and Interior Highlands (Pflieger, 1971; Wiley and Mayden, 1985; Burr and Page, 1986). Some of the darter sister species exhibiting this distribution include *P. stictogaster-P. cymatotaenia* (Burr and Page, 1993), *E. sagitta-E. nianguae* (Page, 1983), and *P. tanasi-P. uranidea* (Etnier, 1976). This biogeographic pattern is hypothesized to have resulted from vicariance caused by the onset of Quaternary glaciers, which dissected a once continuous highland area into disjunct Eastern and Interior Highlands. The phylogenetic prediction from this hypothesis is that populations in the

Eastern and Interior highland areas will be sister taxa and each will be reciprocally monophyletic. Since the vicariance of the once continuous Central Highlands preceded at the onset of the Quaternary, there should be significant genetic divergences between species or populations in the disjunct highland areas.

The recovery of two monophyletic clades with substantial sequence divergence supports a Central Highland vicariance for the origin of the eastern and western populations of *P. evides*. Additionally, the strongly supported monophyly of the northern Ozark and upper Mississippi populations coupled with the very shallow levels of sequence divergence, support a northern Ozark origin for the upper Mississippi populations. The pre-Quaternary hypothesis (Mayden, 1988) for the origin of the upper Mississippi River populations of *P. evides* is not supported in this analysis. The presence of closely related, genetically similar populations of *P.* cf. *evides* in the northern Ozarks and upper Mississippi Drainage is explained by either Sangamonian Interglacial (Pflieger, 1971) or post-Quaternary dispersal (Denoncourt, 1969) from the northern Ozarks. Several other species of freshwater fishes exhibit a similar disjunct distribution between the Ozarks and upper Mississippi and are hypothesized to have re-invaded glaciated regions from Ozark refugia (Burr and Page, 1986).

The hypothesis that the upper Mississippi populations of *P.* cf. *evides* persisted in the Driftless Area of Wisconsin during the Wisconsin Period of

the late Quaternary cannot be supported or refuted by the data collected in this investigation. Potential discrimination of upper Mississippi River populations from the northern Ozark populations will require genetic analysis of multiple locus markers such as allozymes or microsatellites. If appreciable genetic differences are recovered, then a hypothesis of significant temporal reproductive isolation would be supported. The discovery of such divergence would support a Driftless Area refugium (Pflieger, 1971) for these upper Mississippi River populations.

CONCLUSIONS AND RECOMMENDATIONS:

Comparative analysis of cytochrome b sequences from individuals throughout the range of *P. evides* reveals the presence of two monophyletic clades (Figures 1 and 2). This reciprocal monophyly coupled with described morphological variation (Denoncourt, 1969) support the recognition of a new species of *Percina*. This newly discovered taxon encompasses the western populations of *P. evides*. The populations in the upper Mississippi River (St. Croix, Black, and Chippewa rivers) are indistinguishable genetically (Table 2), phylogenetically (Figures 1 and 2), and morphologically (Denoncourt, 1969; Near and Page, unpublished data) from populations in the northern Ozarks. The populations of *P.* cf. *evides* in Minnesota and Wisconsin most likely are the result of post-Quaternary dispersal from a northern Ozark refugium.

Regardless of the taxonomic status of *P.* cf. *evides* populations in Minnesota, these organisms represent a unique and irreplaceable component of Minnesota's ichthyofauna. *P.* cf. *evides* is intolerant of siltation and most often associated with cobble raceways (Hatch, 1985/86). The preference for these habitats may explain the disjunct distribution of these populations from the closely related northern Ozark populations. If multiple locus markers are examined (e.g. allozymes and microsatellites), they may reveal reproductive isolation between these two areas. Recovery of shallow mitochondrial DNA divergences between these two populations should not be used to assume that they are not reproductively isolated. Also, the lack of substantial divergence at mitochondrial genes should not be used as an indicator that individuals from the northern Ozarks can be used as sources for translocation to the upper Mississippi. Again, information from multiple locus markers will be needed to assess such conservation and management decisions.

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Table 1. Collection localaties for specimes of *Percina evides* used in this study. Institutional abbreviations for voucher specimens INHS = Illinois Natural History Survey, UAIC = University of Alabama Ichthyology Collection.

Locality	Drainage	Voucher	Abreviation
Green River, Green Co. KY	Ohio	INHS 64014	GREN
Licking River, Bath Co. KY	Ohio	no voucher	LICK
Clinch River, Clairborne Co. TN	Tennessee	UAIC 10530.17	CLIN
North Fork French Broad River, Transylvania Co. NC	Tennessee	UAIC 7954.16	FRBR
Little Bear Creek, Franklin Co. AL	Tennessee	INHS 45577	LWTN
Tippecanoe River, Fulton Co. IN	Wabash-Ohio	UAIC 10314.14	TIP1
Tippecanoe River, Fulton Co. IN	Wabash-Ohio	UAIC 10314.14	TIP2
St. Croix River, Chisago Co. MN	upper Mississippi	INHS 40661	STCX
Snake River, Kanabec Co. MN	upper Mississippi	INHS 40719	SNKE
Black River, Jackson Co. WI	upper Mississippi	INHS 47466	BLCK
Gasconade River, Gasconade Co. MO	Missouri	no voucher	GASC
White River, Independence Co. AR	White	UAIC 11362.18	WHIT
Current River, Ripley Co. MO	White	UAIC 10786.10	CURR

Table 2. Pairwise sequence divergences at cytochrome b (uncorrected *p*-distance) among all 13 *P. evides* examined. See Table 1 for abreviations.

	GREN	LICK	CLIN	FRBR	LWTN	TIP1	TIP2	STCX	SNKE	BLCK	GASC	WHIT
LICK	0.0044	------										
CLIN	0.0035	0.0026	------									
FRBR	0.0035	0.0026	0.0000	------								
LWTN	0.0035	0.0026	0.0018	0.0018	------							
TIP1	0.0061	0.0053	0.0044	0.0044	0.0044	------						
TIP2	0.0035	0.0026	0.0018	0.0018	0.0018	0.0044	------					
STCX	0.0316	0.0307	0.0281	0.0281	0.0298	0.0289	0.0298	------				
SNKE	0.0307	0.0298	0.0272	0.0272	0.0289	0.0281	0.0289	0.0009	------			
BLCK	0.0316	0.0307	0.0281	0.0281	0.0298	0.0289	0.0298	0.0018	0.0009	------		
GASC	0.0316	0.0307	0.0281	0.0281	0.0298	0.0289	0.0298	0.0018	0.0009	0.0018	------	
WHIT	0.0386	0.0377	0.0351	0.0351	0.0368	0.0342	0.0368	0.0228	0.0219	0.0228	0.0228	------
CURR	0.0404	0.0395	0.0368	0.0368	0.0386	0.0377	0.0386	0.0228	0.0219	0.0228	0.0228	0.0053

Table 3. Uncorrected sequence divergences at cytochrome b for sister species of darters. Data are from Porterfield et al., 1999; Near et al., in press; Near, unpublished.

Sister Species Compared	Cyt b Divergence
Percina uranidea-P. tanasi	0.0061
Percina austroperca-P. kathae	0.0097
Percina maculata-P. pantherina	0.0167
Etheostoma smithi-E. striatulum	0.0254
E. crossopterum-E. olivaceum	0.0342
E. chienense-E. oophylax	0.0377
Ammocrypta beani-A. bifascia	0.0412
Ammocrypta meridiana-A. vivax	0.0597
Percina sciera-P. aurolineata	0.0655
Percina peltata-P. crassa	0.0719
Percina aurantiaca-P. evides	0.0851
Ammocrypta clara-A. pellucida	0.1031
Percina cymatotaenia-P. stictogaster	0.1150

Figure 1. Strict consenscus of three most-parsimonious trees recovered in maximum parsimony analysis (branch-and-bound tree search). Tree length = 355 steps, CI (excluding uninformative characters) = 0.733, RI = 0.840. Numbers above nodes represent percent recovery of the clade in bootstrap analysis (2,000 pseudoreplicates).

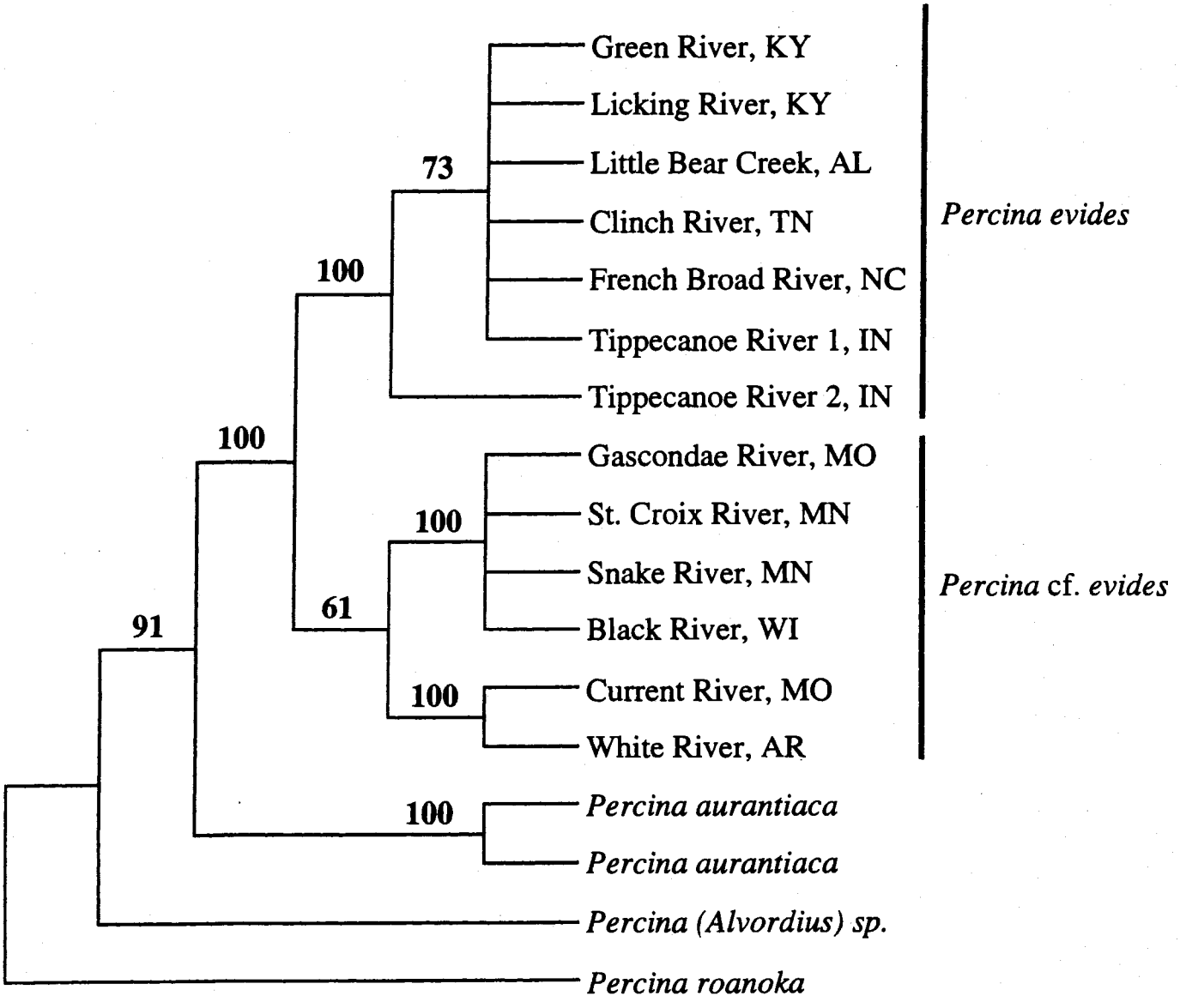

Figure 2. Maximum likelihood inferred topology. Likelihood score (*Ln*) = -3,176.56, gamma shape parameter (α) = 0.1831. Numbers above nodes represent branch lengths, those below nodes represent percent recovery in bootstrap analysis (100 pseudoreplicates).