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Phylogenetic Relationships of *Percina* (Percidae: Etheostomatinae)

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Phylogenetic relationships among species of *Percina* are unresolved. Previous systematic studies of *Percina* have resulted in the recognition of nine subgenera, diagnosed by external morphological characters. Throughout the history of darter taxonomy characters such as large body size, high meristics, drab coloration, and exploitation of a hyperbenthic habitat have been interpreted as pleisiomorphic. Most species of *Percina* exhibit these characters, and have been hypothesized to represent the "primitive" lineage of darters. The hypotheses that each of the polytypic subgenera of *Percina* are monophyletic and that the previously defined primitive characters are pleisiomorphic, have not been investigated with cladistic analyses. In this investigation, complete gene sequences of the mitochondrially encoded cytochrome *b* were collected from a total of 79 individual specimens, representing nine of 10 percid genera and all 40 species of *Percina*. Observed patterns of cytochrome *b* evolution were very similar to those previously reported in other percid fishes. Maximum-parsimony and maximum-likelihood analyses were generally congruent. The majority of subgenera (*Percina*, *Imostoma*, *Cottogaster*, *Swainia*, and *Odontopholis*) were recovered as monophyletic in most analyses. The subgenera *Alvordius*, *Hadropterus*, and *Ericosma* were never recovered as monophyletic; however, monophyly of *Hadropterus* and *Ericosma* could not be rejected in statistical analyses of maximum-likelihood score differences. As a result of these phylogenetic analyses, a novel classification of *Percina* species is proposed. The use of subgenera in *Percina* taxonomy is abandoned in favor of the recognition of monophyletic "species clades." Reconstruction of character evolution on the hypothesized phylogenetic relationships suggest that previously identified pleisiomorphic character states in darters may actually be derived within *Percina*. Hypothesis testing of derived and ancestral traits in darters is complicated by uncertainty in ancestral character state reconstruction. Contributing to the lack of confidence in character optimization are inadequate sampling of *Etheostoma* species, short internal branches on the phylogeny, and a high frequency of character change across the entire diversity of darters.

WITH over 180 species, Percidae is the second largest family of freshwater fishes in North America, and all but three species of North American percids are darters (subfamily Etheostomatinae). Evidence from morphology (Wiley, 1992), behavior (Page, 1985), and mtDNA sequence data (Song et al., 1998) indicate that darters are monophyletic. Relationships among genera, subgenera, and most species, however, remain unresolved.

Percina is the second largest genus (40 species) of darters. Phenetic analyses of external morphological characters have been used to classify species of *Percina* into nine subgenera (Page, 1974, 1981). Most of the characters considered were meristics, morphometric ratios, and pigmentation patterns (Page, 1974, 1981). There is no published cladistic investigation of relationships among species of *Percina* using discretely coded morphological characters (i.e., osteology).

Percina has been hypothesized to include the plesiomorphic darters (Jordan et al., 1930; Page, 1974; Bailey and Etnier, 1988). Unlike other darter genera, species in *Percina* exhibit both hypothesized derived and ancestral characteristics. For example, *Percina lenticula* has been regarded as the most ''primitive'' species of *Percina* (Page, 1974), because it lacks bright breeding colors, has high meristics, and is the largest darter species (Page, 1974; Page and Burr, 1979). On the other hand, *Percina roanoka*

has been hypothesized to be one of the most derived species of *Percina*, because it exhibits bright breeding colors, has low meristics, and is one of the smallest species in the genus (Page, 1974, 1981). Some *Percina* species exhibit a combination of putatively ancestral and derived characteristics. An example is *Percina aurantiaca*, which is among the largest species of darters and has high meristics (Page, 1974, 1983). However, confounding the placement of this species as ancestral among *Percina* is the presence of bright coloration and breeding tubercles, traits identified as being characteristic of derived darter species.

According to current taxonomic placements, the evolution of derived characteristics in *Percina* is restricted to species in the subgenera *Alvordius, Ericosma*, and *Hypohomus*. The presence of derived characteristics in darters is hypothesized to have originated independently within *Percina* and *Etheostoma*, as a result of convergent adaptation to shallow rocky riffles (Page, 1981; Page and Swofford, 1984).

Examination of evolutionary trends within *Percina*, testing hypotheses of convergent evolution, and identifying ancestral and derived traits have not been investigated with cladistic based phylogenetic hypotheses of the genus. Previous investigations of external morphology (Page, 1974) and allozymes (Page and Whitt, 1973a,b) suggest that *Percina* is a monophyletic group. However, estimating relationships among species using these characters is complicated by the lack of discretely coded character state transitions amenable to cladistic analysis. In the absence of phylogenetic hypotheses of relationships, hypotheses concerning the polarity of morphological and ecological trait diversification within darters remain untested. For instance, demonstration that *Percina* species characterized by small benthic forms are derived relative to larger midwater species is impossible without developing phylogenetic hypotheses for these taxa.

This investigation examined the phylogenetic relationships of *Percina* using complete mitochondrially encoded cytochrome *b* gene sequences. Specific hypotheses tested in this study include, the monophyly of the genus *Percina* and the monophyly of each polytypic subgenus. Phylogenetic hypotheses generated in this investigation are used to reassess and illustrate future directions of research concerning the systematics and evolutionary diversification of *Percina*.

Materials and Methods

Collection of DNA sequence data.—Information for all specimens sequenced in this study and cytochrome *b* sequences used from other studies is presented in Material Examined. Nucleic acids were isolated from frozen or ethanol-fixed tissues using standard phenol-chloroform extraction and ethanol precipitation procedures. The complete coding region of the mitochondrial cytochrome *b* gene was PCR amplified using primers and conditions given in Near et al. (2000). Two separate strategies were used to collect DNA sequences from PCR products. First, PCR products were spin filtered using Millipore Ultrafree-MC (30,000 NMWL) units, ligated into pGem-T vector plasmids, and used to transform DH5α-*E. coli*. Isolated cloned plasmids were used as template for cycle sequencing, using primers end labeled with ^{32}P-dATP and the Promega *fmol* sequencing kit. Both strands in each specimen were sequenced using six primers (Song et al., 1998) and two separate clones. Sequencing products were separated by electrophoresis in 6% polyacrylamide/8.3M urea gels and visualized by autoradiography. Second, PCR products were purified using the Qiagen QIAquick kit and used as template for Big Dye (Perkin Elmer) terminator cycle sequencing reactions. Four primers were used to sequence both strands of the purified PCR product (Song et al., 1998). Sequencing products were cleaned of excess nucleotides via centrifugation on sephadex G-50 columns. Sequences were read with an ABI 377 automated sequencer at the W. M. Keck Center for Comparative and Functional Genomics at the University of Illinois Urbana-Champaign. Individual sequence files were edited using EditView ver. 1.0.1 and complete cytochrome *b* sequences were assembled from individual sequencing reactions using the program Sequencher version 3.0 (Gene Codes, Ann Arbor, MI).

Data analysis.—Sequences were aligned using Clustal V (Higgins et al., 1992). Genetic distances and nucleotide composition indices were calculated using PAUP* (Swofford, 2000). Substitution dynamics of cytochrome *b* were investigated by plotting a priori defined character classes against one another (e.g., transitions vs transversions). In particular, differential saturation of purine (A $\leftrightarrow$ G) versus pyrimidine (C $\leftrightarrow$ T) transitions was investigated as in Near et al. (2000). Rate heterogeneity of cytochrome *b* sequence evolution among darter species was investigated using a cladistic relative-rate test (Mindell and Honeycutt, 1990; Near et al., 2000) with *Perca flavescens* as the outgroup for all comparisons. Separate relative-rates tests were executed for all characters and third codon transversions.

Maximum-parsimony analyses were executed using PAUP* (Swofford, 2000). Three different weighting schemes were used: (1) uniform weight for all sites; (2) transversions weighted 5.5:1 to transitions (value of transition transversion ratio determined using maximum likelihood models); and (3) third codon transitions excluded. Tree searches used tree bisection-reconnection (TBR) branch swapping and 100 random addition sequence replicates. For each weighting scheme, bootstrap (2000 pseudoreplicates) analysis was used to assess relative support for inferred monophyletic groupings. Support for nodes based on bootstrap pseudoreplicate scores was qualitatively classified as low (50–59%), moderate (60–69%), and high (70–100%). Decay analysis (Bremer, 1994) was executed only with the uniform-weight analysis and a decay score greater than three was considered high.

The dataset was analyzed to determine the preferred model of sequence evolution using a best-fit criterion (Frati et al., 1997; Cunningham et al., 1998), assessed using hierarchical likelihood ratio tests with a chi-square distribution (Huelsenbeck and Crandall, 1997). A total of 40 progressively complex (parameter-rich) models of sequence evolution were used to calculate log-likelihood scores for a neighbor joining inferred topology. The least complex model which resulted in a significant increase in the likelihood score was chosen. The computer program Modeltest version 2.0 (Posada and Crandall, 1998) was used to calculate likelihood scores and execute likelihood ratio tests. PAUP* was used to execute heuristic tree searches with subtree pruning-regrafting (SPR) branch swapping with 10 replicates using random addition of taxa. The significance of branch lengths in the optimal maximum likelihood topology was assessed by comparing the likelihood ratio to the null hypothesis that the branch has a length of zero as executed in PAUP*. Bootstrap analysis in maximum-likelihood was executed with 100 pseudoreplicates using the preferred model of sequence evolution.

Alternative phylogenetic hypotheses were statistically compared to trees recovered from maximum-likelihood analyses using the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) using bootstrap (1000 replications) with full optimization as executed in PAUP* (Swofford, 2000). The Kishino-Hasegawa (K-H) and modified Templeton (MT) tests commonly used to evaluate alternative phylogenetic hypotheses are inappropriate when the trees compared are a combination of a priori and a posteriori hypotheses (Goldman et al., 2000). Maximum-likelihood was used in separate constrained tree searches to find the best trees that depict the subgenera *Alvordius*, *Hadropterus*, and *Ericosma* as monophyletic lineages. Also, the best tree that represents the placement of *Odontopholis* as the sister taxon of the rest of *Percina* (Burr and Page, 1993) was found with maximum-likelihood constraint tree searches.

Character mapping: Color and habitat.—The hypothesis that the ancestral condition for darters is drab coloration and hyperbenthic habitat was examined by mapping these characters on the phylogeny using parsimony methods. Coding color characters in organisms can be problematic because the homology of specific attributes among the species being investigated must be justified. However, characterization of species as either possessing or lacking bright colors can provide a preliminary assessment of the ancestral condition in a monophyletic clade. This approach has been useful in studying the evolution of dichromatism among all extant avian lineages (Price and Birch, 1996), without particular identification of homologous coloration characters. Bright breeding colors in darters are typically found only in males of *Etheostoma* and *Percina* (Page, 1983). The colors, patterns, and their distribution on the body differ dramatically among darter species and are often diagnostic for species and subgenera (Kuehne and Barbour, 1983; Page, 1983). The goal of this investigation is not to examine the evolution of specific color attributes but only to determine whether the ancestral node of all darters and the common ancestor of *Percina* are optimized as possessing drab coloration as predicted by previous hypotheses (Page, 1974, 1983; Bailey and Etnier, 1988). Each species in the analysis was coded for presence or absence of bright coloration in breeding males (Table 1). Information for this coding was taken from Page (1983), Kuehne and Barbour (1983), Etnier and Starnes (1993), and Jenkins and Burkhead (1994).

The habitat for all of the percid species in the analysis was scored as being midwater, hyperbenthic, or benthic (Table 1). All *Ammocrypta*, *Etheostoma* (except *Etheostoma sagitta*), and *Crystallaria* were scored as benthic (Page 1972, 1983, 1984). Species of *Percina* were scored from information in Page (1972, 1983), Greenberg (1991), Jenkins and Burkhead (1994), and Chipps and Perry (1994). All darters are relatively benthic when compared to truly midwater species. The difference between hyperbenthic and benthic in darters involves species that swim near but off of the benthos and those that ''predominantly sit on the bottom'' (Page and

TABLE 1. SCORING OF BRIGHT BREEDING COLORATION AND HABITAT IN PERCID SPECIES.

Species	Bright breeding colors	Habitat
Percina antesella	absent	benthic
Percina aurantiaca	present	hyperbenthic
Percina aurolineata	absent	hyperbenthic
Percina aurora	absent	benthic
Percina austroperca	absent	hyperbenthic
Percina brevicauda	absent	benthic
Percina burtoni	absent	hyperbenthic
Percina caprodes	absent	hyperbenthic
Percina carbonaria	absent	hyperbenthic
Percina copelandi	absent	benthic
Percina crassa	absent	benthic
Percina cymatotaenia	absent	hyperbenthic
Percina evides	present	benthic
Percina gymnocephala	absent	hyperbenthic
Percina jenkinsi	absent	hyperbenthic
Percina kathae	absent	hyperbenthic
Percina lenticula	absent	hyperbenthic
Percina macrocephala	absent	hyperbenthic
Percina macrolepida	absent	hyperbenthic
Percina maculata	absent	hyperbenthic
Percina nasuta	absent	hyperbenthic
Percina nevisense	absent	benthic
Percina nigrofasciata	absent	hyperbenthic
Percina notogramma	absent	hyperbenthic
Percina oxyrhynchus	absent	hyperbenthic
Percina palmaris	present	benthic
Percina pantherina	absent	hyperbenthic
Percina peltata	absent	benthic
Percina phoxocephala	absent	hyperbenthic
Percina rex	absent	hyperbenthic
Percina roanoka	present	benthic
Percina sciera	absent	hyperbenthic
Percina shumardi	absent	benthic
Percina species	absent	hyperbenthic
Percina stictogaster	absent	hyperbenthic
Percina suttkusi	absent	hyperbenthic
Percina tanasi	absent	benthic
Percina uranidea	absent	benthic
Percina vigil	absent	benthic
Ammocrypta beani	absent	benthic
Ammocrypta meridiana	absent	benthic
Ammocrypta pellucida	absent	benthic
Crystalaria asprella	absent	benthic
Etheostoma aquali	present	benthic
Etheostoma blennioides	present	benthic
Etheostoma camurum	present	benthic
Etheostoma cinereum	present	benthic
Etheostoma flabellare	absent	benthic
Etheostoma gracile	present	benthic
Etheostoma kennicotti	absent	benthic
Ehteostoma sagitta	present	hyperbenthic
Etheostoma vitreum	absent	benthic
Gymnocephalus cernuus	absent	midwater
Perca fluviatilus	absent	midwater
Perca flavescens	absent	midwater
Stizostedion canadense	absent	midwater
Stizostedion vitreum	absent	midwater
Zingel streber	absent	benthic
Romanichthys valsanicola	absent	benthic

Swofford, 1984). Coding of habitat for *Perca*, *Gymnocephalus*, *Stizostedion*, *Zingel*, and *Romanichthys* was based on information in Collette et al. (1977).

The scored discrete characters of coloration and habitat were entered into MacClade 3.0 (Maddison and Maddison, 1992) and mapped using parsimony on the cytochrome *b* inferred phylogenies resulting from uniform-weight parsimony, transversion-weighted parsimony, and maximum likelihood. Characters were treated as unordered and an optimization that found all most-parsimonious reconstructions was used. Distributions of the characters were visually inspected and any differences in optimization among multiple most-parsimonious phylogenies were noted.

RESULTS

Cytochrome b polymorphism.—Among 79 percid OTUs, 520 of 1140 (45.6%) sites were variable, and 450 (39.5%) were parsimony informative. Pairwise sequence polymorphism (*p*-distance) among all OTUs ranged from 0.61–20.96%, with a mean of 13.93% (± 0.15%). The ratio of transitions to transversions ranged between 1.39 and 17.15, with a mean of 3.11 (± 2.02). Typical of patterns previously reported for cytochrome *b* in percid fishes (Song et al., 1998; Porterfield et al., 1999; Near et al., 2000), the majority of polymorphic sites were located at the third codon position (371/520; 71.3%). Interspecific comparisons within *Percina* reveal 463 of 1140 (40.6%) sites were variable, and 383 (33.6%) were phylogenetically informative. The vast majority (86.9%) of phylogenetically informative sites within *Percina* were at the third codon position. Transition:transversion ratios that could be defined among *Percina* species ranged from 1.67–13.00, with a mean of 3.98 (± 1.23).

As discussed in Song et al. (1998), the nucleotide composition in percid cytochrome *b* is biased with a lack of guanine and an overabundance of thymine and cytosine when all codon positions are considered. Second codon sites ex-

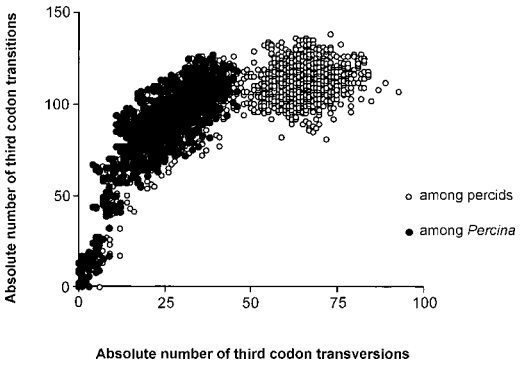

Fig. 1. Plot of absolute numbers of third codon transitions versus third codon transitions. Comparisons among *Percina* species (closed) and among all other comparisons (open) are differentiated.

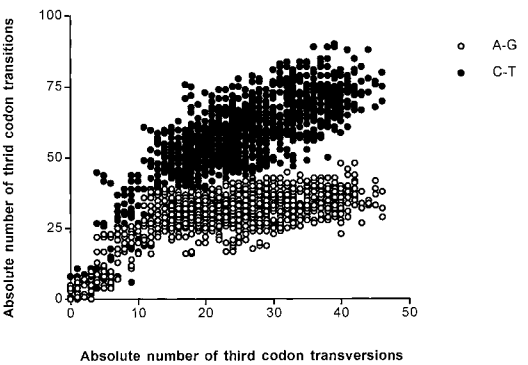

Fig. 2. Plot of third codon purine (open) and pyrimidine (closed) changes versus third codon transitions.

hibit a lack of guanine and an overabundance of thymine. Third codon sites exhibit the most extreme compositional bias with a chronic lack of guanine and an overabundance of cytosine. Phylogenetic analyses are potentially compromised if the composition of nucleotides among taxa vary significantly, resulting in the grouping of unrelated species with similar nucleotide composition (Steel et al., 1993; Lockhart et al., 1994; Naylor and Brown, 1998). The 57 cytochrome *b* sequences sampled from *Percina* exhibited the typical nucleotide biases; however, codon positions among all species did not differ significantly in nucleotide composition (first positions, $\chi^2 = 5.23$, 168 df, $P = 1.00$; second positions, $\chi^2 = 0.91$, 168 df, $P = 1.00$; third positions, $\chi^2 = 91.70$, 168 df, $P = 1.00$). Nucleotide composition biases place a ceiling on the amount of observable cytochrome *b* polymorphism in percids (e.g., Collins et al., 1994), but the recovery of stationarity in *Percina* indicates that nucleotide composition biases has probably not contributed to erroneous phylogenetic inferences.

The effect of nucleotide composition on the observed nucleotide polymorphism within percid cytochrome *b* is evident when plotting the number of transitions versus transversions, as the observed rarity of a particular nucleotide will restrict numbers of observed changes involving the rare character state (Collins et al., 1994). An increasing and linear relationship between pairwise counts of polymorphic nucleotides and evolutionary divergence is expected of sites that are not characterized by multiple substitutions, often referred to as saturation (Moritz et al., 1992). Multiple substitutions were detected in third codon transitions in comparisons among outgroup percid species and among comparisons of *Percina* and other percid species (Fig. 1). Without discriminating among types of transition substitutions, saturation was not detected in *Percina* third codon positions (Fig. 1). However, reflecting the low frequency of guanine, third codon purine (A $\leftrightarrow$ G) transitions plateau much more rapidly than pyrimidine (C $\leftrightarrow$ T) transitions and appear to be experiencing multiple substitutions in most comparisons among *Percina* species (Fig. 2). The pattern of limited guanine at third codon positions and differential saturation between purine and pyrimidine transitions has been demonstrated in percid cytochrome *b* (Near et al., 2000) and cichlid NADH 2 (Kocher and Carleton, 1997).

The parsimony relative-rate tests detected heterogeneity in rates of cytochrome *b* evolution only in comparisons involving *Percina rex*. Unequal amounts of character change were observed for tests that examined all changes and tests restricted to third codon transversions. Unequal rates of change were not detected among the vast majority of parsimony pairwise relative-rate comparisons, indicating that rate heterogeneity is not a prevalent feature of cytochrome *b* evolution in percids.

Phylogenetic analysis of cytochrome b.—Two most-parsimonious trees were recovered in maximum-parsimony analysis using uniform weights for all changes (Fig. 3). *Percina* is monophyletic and is recovered in 99% of bootstrap pseudoreplicates with a decay score of 13. Four of the eight polytypic subgenera are monophyletic, and three of these (*Percina* s.s., *Swainia*, and *Cottogaster*) were supported with high bootstrap and decay scores (Fig. 3). Relationships among species within these monophyletic lineages were relatively well supported with moderate to high bootstrap and decay scores. The subgenus *Im-*

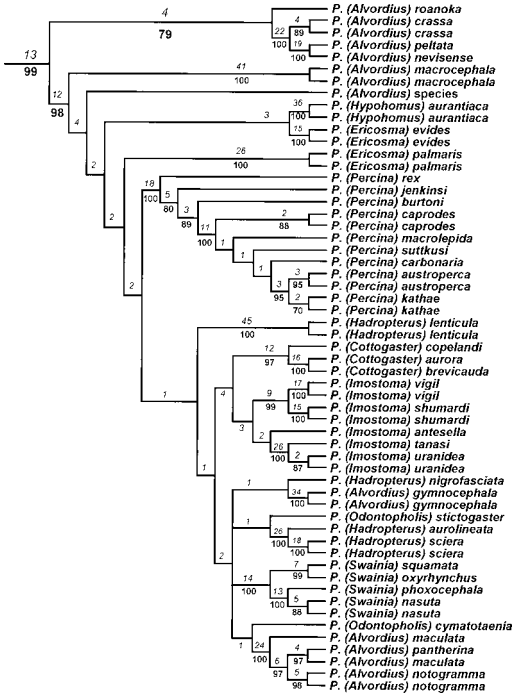

Fig. 3. Strict consensus of two trees recovered in maximum-parsimony analysis using uniform weight for all sites. Tree length = 4214, C.I. (excluding uninformative characters) = 0.196. Numbers in bold represent percent recovery in bootstrap analysis (2000 pseudoreplicates) and numbers in italics are decay scores. Relationships among outgroup species not shown.

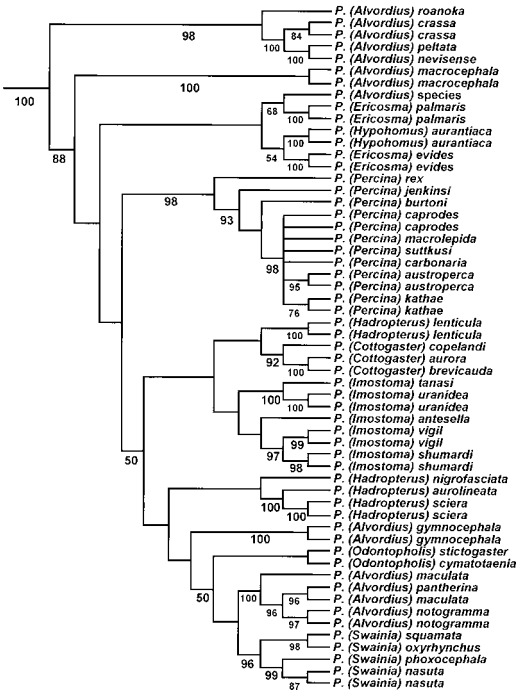

Fig. 4. Strict consensus of two trees recovered in maximum-parsimony analysis where transversions were weighted 5.5:1.0, relative to transitions. Tree length = 8,316.5, C.I. (excluding uninformative characters) = 0.228. Numbers at nodes represent percent recovery in bootstrap analysis (2,000 pseudoreplicates). Relationships among outgroup species not shown.

ostoma was recovered as monophyletic with a high decay score; however, it was not recovered in 50% or greater of the bootstrap pseudoreplicates. The subgenera *Imostoma* and *Cottogaster* are sister taxa, supported with a high decay score. The subgenera *Ericosma*, *Hadropterus*, *Odontopholis*, and *Alvordius* were not monophyletic in the uniform-weight maximum-parsimony analysis. The type species of *Ericosma*, *P. evides*, was recovered as the sister taxon of *P. aurantiaca*, which is the only species in the subgenus *Hypohomus*. This node was not recovered in > 50% of bootstrap pseudoreplicates but has a relatively high decay score (Fig. 3).

Perhaps the most interesting result of the phylogenetic analysis is the apparent non monophyly of *Alvordius*, the largest *Percina* subgenus. Two clades were recovered at the base of the *Percina* tree, and both were supported with high bootstrap pseudoreplicate recovery and decay scores (Fig. 3). One of these clades contains four of five Atlantic Slope *Alvordius* (*P. roanoka*, *P. crassa*, *P. peltata*, and *P. nevisense*). The other clade contains all remaining species of *Percina*. Across the maximum-parsimony tree *Alvordius* species occur in five separate clades (Fig. 3). Two of these clades contain single species (*P.* sp. and *P. macrocephala*). The third clade is the basal grouping of four Atlantic Slope species discussed above. The fourth clade is a weakly supported *P.* (*Hadropterus*) *nigrofasciata*–*P.* (*Alvordius*) *gymnocephala* lineage. The fifth clade of *Alvordius* contains the type species of the subgenus, *P. maculata*, which is not recovered as monophyletic (Fig. 3). *P. maculata* from the Wabash is the sister taxon of *P. pantherina*. This clade is sister to *P. notogramma* of the Atlantic Slope. This larger clade is sister to the *Percina maculata* sampled from the Kentucky River Drainage (Fig. 3). All other species of *Percina* that were sampled with more than one specimen are recovered as monophyletic.

Two most-parsimonious trees were recovered in transversion-weighted maximum-parsimony analysis (Fig. 4). Much of the topology of these trees are congruent with trees recovered in the uniform-weight maximum-parsimony analysis (Fig. 3). The subgenera *Percina* s.s., *Swainia*, and

Cottogaster were recovered as monophyletic with high bootstrap pseudoreplicate scores (Fig. 4). The subgenus *Imostoma* was recovered as monophyletic but is not supported in > 50% of bootstrap pseudoreplicates. Different from the uniform-weight maximum-parsimony analysis, the transversion-weighted maximum-parsimony analysis recovered *Odontopholis* as monophyletic (Fig. 4); however, the clade is not recovered in bootstrap analysis. Differing from the uniform-weight analysis, the placement of *P. lenticula* as the sister taxon of *Cottogaster* confounds the monophyly of *Cottogaster* and *Imostoma* recovered in the uniform-weight analysis (Fig. 3), but this clade is not supported bootstrap score of 50% or greater. The subgenera *Alvordius*, *Hadropterus*, and *Ericosma* were not monophyletic (Fig. 4). Similar to the uniform-weight maximum-parsimony analysis, species of *Alvordius* were grouped into five separate clades (Fig. 4). Unlike the uniform-weight parsimony analysis, the transversion-weighted parsimony analysis supports the *P. evides* + *P. aurantiaca* clade with > 50% bootstrap support. Also the Mobile Basin endemic species, *P.* (*Alvordius*) species and *P.* (*Ericosma*) *palmaris* were recovered as sister species with a moderate bootstrap pseudoreplicate score (Fig. 4).

The maximum parsimony analysis ignoring all third codon position transitions recovered 15,534 most-parsimonious trees. The strict consensus of these trees (Fig. 5) exhibited a remarkable degree of resolution, considering the large number of trees recovered. The consensus tree (Fig. 5) is generally congruent with uniform-weight (Fig. 3) and transversion-weighted (Fig. 4) maximum-parsimony analyses. The subgenera *Percina*, *Cottogaster*, *Swainia*, and *Odontopholis* were recovered as monophyletic (Fig. 5). Unlike the transversion-weighted maximum-parsimony analysis (Fig. 4), *Odontopholis* is recovered in > 50% of bootstrap pseudoreplications. Bootstrap pseudoreplicate scores were lower for the subgenera *Percina*, *Swainia*, and *Cottogaster* than seen in uniform-weight and transversion-weighted parsimony analyses (Figs. 3–4). The subgenera *Alvordius*, *Hadropterus*, and *Ericosma* were not recovered as monophyletic, and species of *Alvordius* were again distributed among five separate clades (Fig. 5). *Imostoma* was not recovered as monophyletic; species of this group were collapsed into a polytomy along with *Cottogaster* and *P. lenticula*, and *P. antesella* was recovered as the sister taxon of *P. nigrofasciata* (Fig. 5).

The model of sequence evolution selected with Akaike Information Criterion (AIC) was the general time reversible (GTR+I+Γ), with

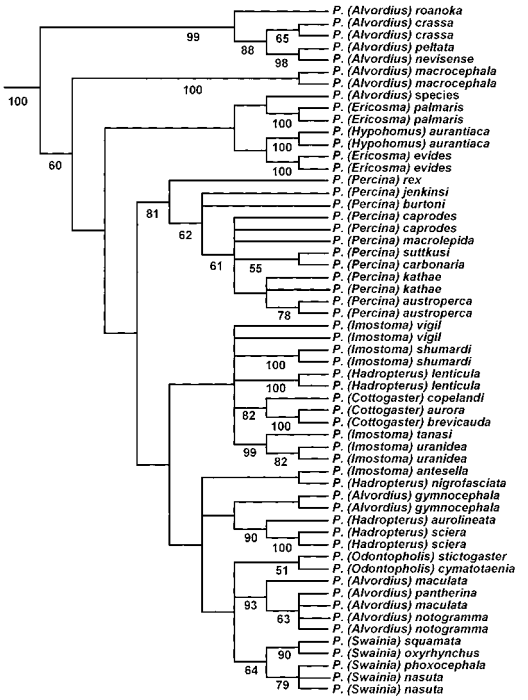

Fig. 5. Strict consensus of 15,534 trees recovered in maximum-parsimony analysis ignoring third codon transitions. Tree length = 1273, C.I. (excluding uninformative characters) = 0.245. Numbers at nodes represent percent recovery in bootstrap analysis (2000 pseudoreplicates). Relationships among outgroup species not shown.

the proportion of invariable sites (0.5161) and gamma shape parameter ($\alpha = 0.9193$) estimated from initial maximum-parsimony inferred trees. The rate variables for the GTR model were R(a) [A–C] = 1.5839, R(b) [A–G] = 25.0919, R(c) [A–T] = 1.2103, R(d) [C–G] = 1.8717, R(e) [C–T] = 11.4793, R(f) [G–T] = 1.0000 with frequency of nucleotides A = 0.2506, C = 0.3483, G = 0.1265, and T = 0.2746. Maximum-likelihood tree search recovered a single tree that is characterized by exhibiting short internodal branch lengths, relative to terminal branches (Fig. 6). Identical to the three maximum-parsimony analyses (Figs. 3–5), *Percina* is monophyletic and a basal split between *P. roanoka*, *P. peltata*, *P. nevisense*, and *P. crassa* versus all other species of *Percina* is present. Each of the branches leading to these two clades is comparatively longer than all other internode branches (Fig. 6). The subgenera *Percina*, *Swainia*, *Cottogaster*, *Imostoma*, and *Odontopholis* are monophyletic in the maximum-likelihood tree (Fig. 6). Also, *Cottogaster* and *Imostoma* are sister taxa, and this clade is sister to *P. len-*

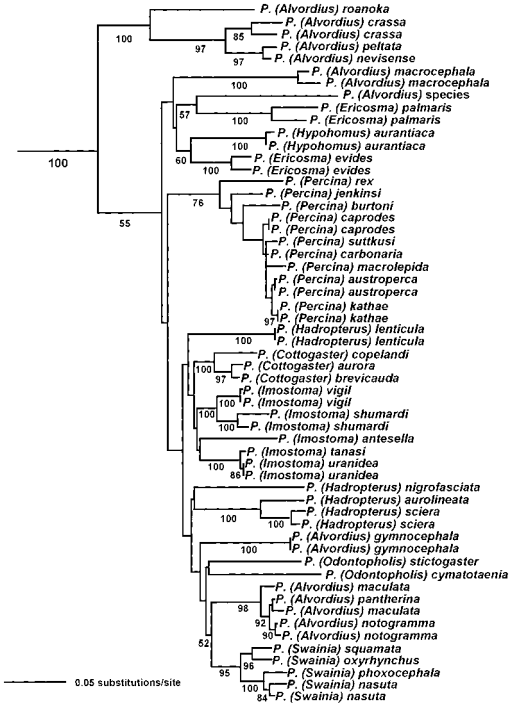

Fig. 6. Maximum-likelihood inferred topology using GTR+Γ+I model of sequence evolution. *ln* L = −18,457.40, α = 0.9193, and I = 0.5161. Numbers at nodes represent percent recovery in bootstrap analysis (100 pseudoreplicates). Relationships among outgroup species not shown.

ticula. The *P. evides* + *P. aurantiaca* clade received moderate bootstrap pseudoreplicate support (Fig. 6). As in the maximum-parsimony analyses, the subgenera *Alvordius*, *Hadropterus*, and *Ericosma* were not monophyletic, and species of *Alvordius* were distributed among five separate clades (Fig. 6).

Examination of alternative phylogenetic hypotheses.—The SH maximum-likelihood test did not reject the monophyly of *Ericosma* and *Hadropterus* (Table 2), despite recovery of these subgenera as nonmonophyletic in the maximum-likelihood tree search (Fig. 6). The hypothesis that *Odontopholis* (*P. stictogaster* and *P. cymatotaenia*) is the sister taxon to the remaining species of *Percina* (Burr and Page, 1993) was rejected (Table 2). Also the hypothesis that *Alvordius* is monophyletic was rejected (Table 2), and this hypothesis had the lowest maximum-likelihood score among all topologies examined.

Character mapping: Color and habitat.—The optimization of color condition on the phylogenies differed between the uniform-weight parsimony and the other two analyses (Table 3). The ancestral nodes for darters and *Percina* were each optimized as bright colors absent, but the optimization was equivocal on the transversion-weighted parsimony and maximum likelihood trees (Table 3). The optimization on the uniform-weight parsimony tree is dependent on the relationship that darters comprise two monophyletic lineages, *Percina* versus all other darter species (Fig. 3). The equivocal optimization on the trees from the other two analyses is the result of not recovering *Etheostoma*, *Crystallaria*, and *Ammocrypta* as a monophyletic clade (Figs. 4, 6). Regardless of the trees used for optimization, coloration exhibited a large number of changes among all percid species and within *Percina* (Table 3). In all analyses, the habitat occupied was optimized as benthic for the common ancestors of all darters and *Percina*. Similar to color, there were many changes in habitat observed among all percids and within *Percina* (Table 3).

Discussion

Several aspects of the taxonomy and evolutionary biology of *Percina* merit reexamination in light of the hypotheses of phylogenetic relationships inferred from mtDNA sequence data. The phylogenetic trees offer several surprising results with regards to the systematics of *Percina*. The largest subgenus, *Alvordius*, is not mono-

Table 2. Summary of SH Tests of Alternative Topologies. A significant difference between topologies is indicated with an asterisk.

	ln L	*ln* L diff.	No. trees[a]	*P*
Maximum-likelihood (Fig. 6)	−18,457.40	best	—	—
Ericosma monophyletic[b]	−18,465.14	7.74	1	0.256
Hadropterus monophyletic[b]	−18,469.94	12.54	1	0.172
Odontopholis basal lineage[b]	−18,484.63	27.23	1	0.030*
Alvordius monophyletic[b]	−18,528.88	71.48	1	≪0.001*

[a] Number of trees recovered in constraint searches.
[b] Maximum-likelihood tree recovered from constraint search.

TABLE 3. RESULTS OF CHARACTER OPTIMIZATION FOR HABITAT AND COLOR ON TREES RECOVERED FROM DIFFERENT PHYLOGENETIC ANALYSES OF CYTOCHROME *B* SEQUENCES. Character state optimized at ancestral node and number of character state changes is given for all darters and *Percina*.

	Bright breeding coloration			
	Ancestral node for all darters		Ancestral node for *Percina*	
Analysis	Optimization	No. of changes	Optimization	No. of changes
Uniform-weight parsimony (Fig. 3)	Absent	8	Absent	3
Weighted parsimony (Fig. 4)	Equivocal	8	Equivocal	3
Maximum-likelihood (Fig. 6)	Equivocal	8	Equivocal	3
	Habitat			
	Ancestral node for all darters		Ancestral node for *Percina*	
Analysis	Optimization	No. of changes	Optmization	No. of changes
Uniform-weight parsimony (Fig. 3)	Benthic	7	Benthic	4
Weighted parsimony (Fig. 4)	Benthic	8	Benthic	5
Maximum-likelihood (Fig. 6)	Benthic	7	Benthic	4

phyletic, with species distributed in five separate clades. Constraint trees that depicted *Alvordius* as monophyletic were rejected in statistical comparisons to optimal topologies recovered in maximum-likelihood analyses (Table 2). Previous investigators have questioned the validity of a monophyletic *Alvordius* (Suttkus et al., 1994) but did not rely on phylogenetic methods to reach this conclusion. The subgenera *Ericosma* and *Hadropterus* were not monophyletic in any of the maximum-parsimony and maximum-likelihood analyses (Figs. 3–6). However, in statistical comparisons between constraint and optimal trees, the monophyly of *Ericosma* and *Hadropterus* could not be rejected (Table 2).

An interesting pattern of monophyly of subgenera is revealed when diagnostic characters of the subgenera, as described in Page (1974), are considered with reference to the hypothesized phylogenetic relationships. Some subgenera were nearly resolved as monophyletic groups regardless of weighting scheme or optimality criterion chosen. These were *Percina* s.s., *Imostoma*, *Odontopholis*, *Swainia*, and *Cottogaster* (Figs. 1–4). Two subgenera that were not consistently recovered as monophyletic are *Odontopholis*, which is not monophyletic in the uniform-weight analysis (Fig. 3), and *Imostoma*, which is not monophyletic in the parsimony analysis ignoring third codon transitions (Fig. 5). All of these subgenera, except *Cottogaster*, are the only intrageneric groups that are diagnosed with discrete apomorphic character states not found in any other species of *Percina* (Page, 1974; Etnier and Starnes, 1993). For example, the subgenus *Percina* s.s. is diagnosed by a bulbous snout (supported by collagen; A. M. Simons, pers. com.), *Imostoma* is diagnosed by an elongated anal fin in males, *Odontopholis* is diagnosed by the presence of a caudal keel covered with strongly toothed scales in males and the absence of modified scales along the midline of the belly, and *Swainia* is diagnosed by the presence of an elongated snout (profile defined by the premaxilla; A. M. Simons pers. com.). Other characters that have been offered as diagnostic for *Percina* subgenera are either polymorphic among species within a particular subgenus or are found in species outside of the subgenus (Page, 1974).

A character that is given as diagnostic for more than one subgenus is the absence of a premaxillary frenum in *Imostoma* and *Cottogaster*. Page (1974) hinted at a relationship between these two subgenera, implying a single evolutionary origin of this character in *Percina*. This conclusion was challenged on the basis that protractile premaxillaries, as indicated by the lack of a frenum, occur in species from other darter genera and are probably independently derived in *Imostoma* and *Cottogaster* (Suttkus et al., 1994). All of the phylogenetic analyses, except transversion-weighted maximum-parsimony, recovered a sister taxon relationship between *Imostoma* and *Cottogaster* (Figs. 3, 5–6), supporting the hypothesis that the absence of a premaxillary frenum has a single evolutionary origin in *Percina*.

The development of a subgeneric classification of *Percina* based on the phylogenetic hypotheses inferred from cytochrome *b* would require the recognition of several new monotypic groups. The only exception is the Atlantic slope species, *P. roanoka*, *P. peltata*, *P. nevisense*, and *P. crassa*, which form a strongly supported monophyletic clade that could potentially be recognized as a subgenus. In contrast, several *Alvordius* species do not group strongly with any other *Percina* lineages and would necessitate the

recognition of at least four new monotypic subgenera, which would prove to be extremely difficult, if not impossible, to diagnose using external morphological features. Using the current subgeneric arrangement as a null hypothesis to compare with the phylogenetic relationships inferred from cytochrome *b,* 74.4% of all *Percina* species are assigned to polytypic subgenera that are either recovered as monophyletic or monophyly cannot be rejected in statistical tests. What is the ideal strategy to classify species assigned to *Alvordius* that represents monophyletic lineages? One option is to abandon the subgenus as a level of taxonomic recognition in *Percina*. To recognize phylogenetic groupings in *Percina*, I propose that polytypic monophyletic lineages recovered in phylogenetic analyses with appreciable branch support be given ''species clade'' names (Table 4). The continued use of subgenera in *Percina* taxonomy will only perpetuate the recognition of artificial groups of species, or at best be modified to include several nondiagnosable and arbitrarily defined monotypic groups. Replacing the subgenus with clades in *Percina* permits the recognition of monophyletic lineages, facilitates modification of classification with future cladistic analyses, and does not require that all species be placed in a species clade.

The analysis of cytochrome *b* DNA sequences has provided the first cladistic hypothesis of relationships within *Percina* based exclusively on discrete characters. Previous investigations had utilized meristic, morphometric, and color/pigmentation characters to infer relationships of *Percina* (Page, 1974, 1981). These efforts relied on clustering algorithms which concurrently considered both discrete and continuous variables. No cladistic analysis using coded osteological and myological characters in *Percina* have been published. Until the phylogenetic utility of such characters is investigated, hypotheses of relationships inferred from mtDNA sequence data cannot be considered to conflict with morphology-based hypotheses. Because previous ideas of *Percina* relationships were based on empirical foundations that did not involve cladistic analyses, the cytochrome *b* gene tree should be accepted as the best available hypothesis of both phylogenetic and taxonomic relationships (Brower et al., 1996).

In the history of percid taxonomy and systematics, *Percina* has long been considered the most ''primitive'' darter genus. The reasons for this assignment are *Percina* contains the largest darter species and that most *Percina* species are hyperbenthic, relative to the other darter genera. *Percina* has been interpreted as being an evolu-

TABLE 4. PROPOSED CLASSIFICATION OF *Percina*.

Percina peltata clade
- *P. crassa*
- *P. nevisense*
- *P. peltata*
- *P. roanoka*

Percina caprodes clade
- *P. austroperca*
- *P. burtoni*
- *P. caprodes*
- *P. carbonaria*
- *P. jenkinsi*
- *P. kathae*
- *P. macrolepida*
- *P. rex*
- *P. suttkusi*

Percina copelandi clade
- *P. aurora*
- *P. brevicauda*
- *P. copelandi*

Percina shumardi clade
- *P. antesella*
- *P. shumardi*
- *P. tanasi*
- *P. uranidea*
- *P. vigil*

Percina sciera clade
- *P. aurolineata*
- *P. sciera*

Percina cymatotaenia clade
- *P. cymatotaenia*
- *P. stictogaster*

Percina maculata clade
- *P. maculata*
- *P. notogramma*
- *P. pantherina*

Percina phoxocephala clade
- *P. nasuta*
- *P. oxyrhynchus*
- *P. phoxocephala*
- *P. squamata*

Species not classified into clades
- *P. aurantiaca*
- *P. evides*
- *P. gymnocephala*
- *P. lenticula*
- *P. macrocephala*
- *P. nigrofasciata*
- *P. palmaris*
- *P. species*

tionary intermediate between much larger, drab colored, midwater species of nondarter percids (e.g., *Perca* and *Stizostedion*) and smaller, benthic, brightly colored species of *Etheostoma*. As revealed in previous phylogenetic studies using allozymes and cytochrome *b* sequences, the phylogenetic relationships among the darter genera are unresolved (Wood and Mayden, 1997; Song et al., 1998; Near et al., 2000). Therefore, there is no cladistic basis to presume that *Percina* is the sister taxon of the remaining darter genera, an expectation of the hypothesis that *Percina* exhibits ancestral conditions of darters.

Two features that have been hypothesized to be derived in darters are the benthic habitat and bright breeding coloration (Page and Swofford, 1984; Bailey and Etnier, 1988). The expectation of this hypothesis is that the hyperbenthic habitat and drab breeding coloration should be optimized as ancestral for all darters, as well as *Percina*. Bright coloration is optimized as absent in the common ancestor for both all darters and *Percina* using the uniform-weight parsimony tree (Fig. 3); however, the optimization is equivocal on the transversion-weighted parsimony and maximum likelihood trees (Table 3). The differences in optimization between these two sets of trees is based entirely on topological differences involving the relationships among darter genera and not among species of *Percina*. This result does not provide confidence in the determination of ancestral character states for coloration in darters. The optimization of coloration is problematic because of the lack of *Etheostoma* species sampled, the presence of short and poorly supported internal nodes relating the genera of darters, and the high frequency of character change observed in coloration (Table 3).

The lack of confidence in the optimization of coloration does not detract from the result that the brightly colored species of *Percina* (*P. roanoka*, *P. aurantiaca*, *P. evides*, and *P. palmaris*) are placed on the phylogeny close to the common ancestor of *Percina*, relative to most drab colored species (Figs. 3–6). The hypothesis that brightly colored species of *Percina* (i.e., *P. roanoka*) are derived, relative to drab colored species (Page and Swofford, 1984) is not supported by the basal placement of these taxa on the *Percina* phylogeny.

The premise that the hyperbenthic habitat is ancestral for darters is not supported by character optimization. The common ancestral node for both all darters and *Percina* is optimized as benthic (Table 3). The most apical species and nodes in the *Percina* phylogeny are optimized as hyperbenthic. Interestingly, a relatively derived benthic clade of *Percina* (the *P. copelandi* and *P. shumardi* clades) seem to have evolved benthicity from a hyperbenthic common ancestor.

The development of a hypothesis of phylogenetic relationships among species of *Percina* has revealed that the currently accepted taxonomy does not adequately recognize monophyletic groups and hypotheses concerning the ancestral condition of darters need reassessment. Because of their taxonomic and biologic diversity, the darters provide a system useful in understanding the processes of speciation, the historical biogeography of North America (Wiley and Mayden, 1985; Strange and Burr, 1997), and evolution of life history and reproductive strategies (Page, 1985; Turner et al., 1996). Paramount to investigations of evolutionary diversification of darters is a well-resolved and relatively robust species-level phylogenetic hypothesis. Analysis of cytochrome *b* gene sequence data has provided the first step in attaining this goal in *Percina* and will potentially serve a similar role in the diversity of percids as a whole (Song et al., 1998; Porterfield et al., 1999; Near et al., 2000).

Material Examined

Voucher specimens (if available) are deposited in the Illinois Natural History Survey (INHS) or the University of Alabama Ichthyology Collection (UAIC). Collection localities (drainage), museum catalog number, TJN tissue catalog number, reference (if appropriate), and GenBank accession numbers are as follows: *Percina crassa*, Lynches Creek (Atlantic Ocean), Lancaster County, South Carolina, UAIC 10014.11, TJN 135, AF386593, Cape Fear River (Atlantic Ocean) Harnett County, North Carolina, no voucher, TJN 542, AF386594; *P. roanoka*, Blackwater River (Roanoke River-Atlantic Ocean) Franklin County, Virginia, INHS 64359, TJN 76, AF386597; *P. peltata*, South Anna River (Atlantic Ocean), Louisa County, Virginia, UAIC 9825.11, TJN 132, AF386595; *P. nevisense*, Blackwater River (Roanoke River-Atlantic Ocean), Franklin County, Virginia, no voucher, TJN 1271, AF386596; *P. macrocephala*, Little River (Tennessee River), Blount County, Tennessee, no voucher, TJN 184, AF386591, French Creek (Allegheny River-Ohio River), Crawford County, Pennsylvania, INHS 39196, TJN 318, AF386592; *P.* species, Crooked Creek (Tallapoosa River-Mobile Bay), Clay County, Alabama, INHS 37616, TJN 192, AF386588; *P. palmaris*, Hillabee Creek (Tallapoosa River-Mobile Bay), Tallapoosa County, Alabama, INHS 38631, TJN 185,

AF386583, Conasauga River (Coosa River-Mobile Bay), Polk County, Tennessee, INHS 41803, TJN 435, AF386584; *P. aurantiaca*, Emory River (Tennessee River), Morgan County, Tennessee, INHS 64349, TJN 88, AF386579, INHS 38299, TJN 341, AF386580; *P. evides*, Green River (Ohio River), Green County, Kentucky, INHS 64014, TJN 79, AF375938, Black River (Mississippi River), Jackson County, Wisconsin, INHS 47466, TJN 1134, AF375951; *P. rex*, Roanoke River (Atlantic Ocean), UAIC 7932.15, TJN 147. AF386556; *P. jenkinsi*, Conasauga River (Coosa-Mobile Bay), Whitfield County, Georgia, UAIC 11680.01, TJN 160, AF386555; *P. burtoni*, Buffalo River (Tennessee River), Wayne County, Tennessee, INHS 38531, TJN 335, AF386554; *P. caprodes*, Lake Wawasee (Lake Michigan), Kosciusko County, Indiana, INHS 68983, TJN 349, AF386550, Song et al. 1998, AF045354; *P. macrolepida*, South Fork San Gabriel River (Gulf of Mexico), Williamson County, Texas, UAIC 11681.01, TJN 158, AF386552; *P. suttkusi*, Bogue Chitto River (Pearl River-Gulf of Mexico), UAIC 10466.12, TJN 159, AF386551; *P. carbonaria*, Colorado River (Gulf of Mexico), Travis County, Texas, UAIC 11412.18, TJN 309, AF386553; *P. austroperca*, Big Escambia Creek (Escambia River-Gulf of Mexico), Escambia County, Alabama, UAIC 9993.19, TJN 129, AF386546, Escambia River (Gulf of Mexico), INHS 34844, TJN 220, AF386547; *P. kathae*, Hillabee Creek (Tallapoosa River-Mobile Bay), Tallapoosa County, Alabama, INHS 38632, TJN 166, AF386548, Sipsey Fork (Black Warrior-Mobile Bay), Winston County, Alabama, INHS 48684, TJN 1242, AF386549; *P. lenticula*, Leaf River (Pascagoula-Gulf of Mexico), Jones County, Mississippi, INHS 38773, TJN 211, AF386585, TJN 212 AF386586; *P. copelandi*, Green River (Ohio River), Green County, Kentucky, no voucher, TJN 89, AF386568; *P. aurora*, Leaf River (Pascagoula River-Gulf of Mexico), Lamar County, Mississippi, UAIC 10458.14, TJN 149, AF386566; *P. brevicauda*, Cahaba River (Alabama River-Mobile Bay), Bibb County, Alabama, UAIC 11679.01, TJN 148, AF386567; *P. tanasi*, French Broad River (Tennessee River), Knox County, Tennessee, no voucher, TJN 485, AF386578; *P. uranidea*, Current River (White River), Clay County, Arkansas, UAIC 7998.21, TJN 155, AF386576, TJN 156, AF386577; *P. antesella*, Conasauga River (Coosa-Mobile Drainage), Bradley County, Tennessee, no voucher, TJN 191, AF386587; *P. vigil*, Bayou de Chien (Mississippi River), Hickman County, Kentucky, no voucher, TJN 90, AF386569, Haley Creek (Tennessee River), Henderson County, Tennessee, INHS 38814, TJN 351, AF386570; *P. shumardi*, Mississippi River (Gulf of Mexico), Jo Daviess County, Illinois, INHS 43065, TJN 527, AF386571, Big Muddy River (Mississippi River), Jackson County, Illinois, INHS 42544, TJN 479, AF386572; *P. nigrofasciata*, Burnt Corn Creek (Escambia River-Gulf of Mexico), Escambia County, Alabama, INHS 38110, TJN 176, AF386590; *P. aurolineata*, Cahaba River (Alabama River-Mobile Bay), Bibb County, Alabama, UAIC 10511.20, TJN 128, AF386575; *P. sciera*, Strong River (Pearl River-Gulf of Mexico), Simpson County, Mississippi, INHS 38604, TJN 345, AF386573; Embarrass River (Wabash River-Ohio River), Coles County, Illinois, no voucher, TJN 106, AF386574; *P. gymnocephala*, Fox Creek (New River-Ohio River), Grayson County, Virginia, UAIC 9848.17, TJN 154, AF386581, Little River (New River-Ohio River), Floyd County, Virginia, INHS 49734, TJN 1224, AF386582; *P. stictogaster* Song et al. 1998, AF045355; *P. cymatotaenia*, Gasconade River (Missouri River), Laclede County, Missouri, no voucher, TJN 125, AF386589; *P. maculata*, Song et al. 1998, AF045353, Dismal Creek (Kaskaskia River-Mississippi River), Fayette County, Illinois, no voucher, TJN 75, AF386557; *P. pantherina*, Big Eagle Creek (Red River), no voucher, TJN 325, AF386558; *P. notogramma*, South Anna River (York River-Atlantic Ocean), INHS 34991, TJN 209, AF386559, TJN 210, AF386560; *P. squamata*, Daddys Creek (Tennessee River), UAIC 11420.01, TJN 305, AF386564; *P. oxyrhynchus*, Licking River (Ohio River), UAIC 8421.26, TJN 170, AF386565; *P. phoxocephala*, Embarrass River (Wabash River-Ohio River), no voucher, TJN 77, AF386563; *P. nasuta*, Middle Fork Little Red River (White River), Searcy County, Arkansas, no voucher, TJN 235, AF386561, Ouachita River (Red River), Montgomery County, Arkansas, no voucher, TJN 290, AF386562; *Etheostoma gracile*, Dismal Creek (Wabash River-Ohio River), Fayette County, Illinois, no voucher, TJN 704, AF386538; *E. sagitta*, Poor Fork Creek (Cumberland River), Letcher County, Kentucky, no voucher, TJN 697, AF386542; *E. blennioides*, West Fork Pond River (Green River-Ohio River), Christian County, Kentucky, INHS 32700, TJN 756, AF386539, *E. vitreum*, Blackwater River (Roanoke River-Atlantic Ocean), Franklin County, Virginia, INHS 64357, TJN 976, AF386540; *E. exile*, Lake Andrusia (Mississippi River), Beltrami County, Minnesota, INHS 39506, TJN 262, AF386541; *E. kennicotti*, Poor Fork Creek (Cumberland River), Letcher County, Kentucky, no voucher, TJN 118, AF386543; *E. flabellare*, Blackwater River (Roanoke River-Atlantic Ocean), Franklin County, Virginia, no voucher, TJN 912, AF386544; *E. cinereum*, Rockcastle River (Cumberland River), Rockcastle County, Kentucky, no

voucher, TJN 689, AF386545; *E. camurum*, Middle Fork of the Vermilion River (Wabash River-Ohio River), Vermilion County, Illinois, no voucher, TJN 1203, AF386536; *E. aquali*, Buffalo River (Tennessee River), Lewis County, Tennessee, no voucher, TJN 68, AF386537; *Crystallaria asprella*, Cahaba River (Alabama-Mobile Bay), Bibb County, Alabama, no voucher, JCP 50, AF386534; Wood and Raley, 2000, AF099881; *Ammocrypta pellucida*, Near et al., 2000, AF183943; *A. beani*, Strong River (Pearl River-Gulf of Mexico), Simpson County, Mississippi, INHS 38611, TJN 314, AF386535, *A. meridiana*, Near et al., 2000, AF183942; *Stizostedion canadense*, Mississippi River (Gulf of Mexico), Jo Daviess County, Illinois, INHS 43066, TJN 512, AF386603; *S. vitreum*, Mississippi River (Gulf of Mexico), Rock Island County, Illinois, no voucher, TJN 312, AF386602; *Zingel streber*, Turiec River, Slovakia, no voucher, TJN 1462, AF386601; *Romanichthys valsanicola*, Song et al., 1998, AF045361; *Perca flavescens*, Lake Andrusia (Mississippi River), Beltrami County, Minnesota, INHS 39508, TJN 261, AF386600; *P. fluviatilus*, Lake Windermere, England, UK, INHS 33646, TJN 794, AF386599; *Gymnocephalus cernuus*, Brunnsviken Bay, Uppland Sweden, UAIC 8533.01, TJN 151, AF386598.

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