

Phylogenetic Relationships of Major Clades of Catostomidae (Teleostei: Cypriniformes) as Inferred from Mitochondrial SSU and LSU rDNA Sequences

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Suckers (Family Catostomidae) are holarctic in distribution and include 76 recent species in 14 genera, with 13 genera and 75 species occurring in North and Central America and Siberia. Although this group constitutes a significant component of many aquatic ecosystems, most historic systematic effort has been either alpha- or limited beta-level studies focusing on the two largest tribes within the family, the Catostomini and the Moxostomatini. A recent phylogenetic study based on morphological, biochemical, and early life history characters has advanced current understanding of relationships among catostomid fishes. To further examine phylogenetic relationships among basal lineages of catostomids, we sequenced the entire mitochondrial (mt) SSU and LSU rRNA genes from genera representing all subfamilies and tribes within Catostomidae. Phylogenetic analysis of gene sequences yielded monophyletic Catostomidae, Ictiobinae, and Catostominae and para- or polyphyletic Cycleptinae, with *Myxocyprinus* as the basal-most taxon and *Cycleptus* as either the next most-basal taxon or the taxon basal to the Catostominae. Relationships within the Catostominae were generally consistent with those proposed in the above-noted recent phylogenetic study although *Thoburnia* and *Hypentelium* were either a clade sister to or a grade group relative to *Moxostoma* and *Scartomyzon*. In all trees, *Scartomyzon* was paraphyletic and embedded within *Moxostoma*. Phylogenetic affinities of *Erimyzon* and *Minytrema* varied depending on data set and character weighting scheme employed. To better reflect phylogenetic relationships resolved in this extensive analysis, we propose the following changes to the classification of catostomids: formation of the new subfamily Myxocyprininae, containing *Myxocyprinus* from China; restriction of the Cycleptinae to the two species of *Cycleptus* from North America; restriction of the tribe Moxostomatini to *Moxostoma*

and *Scartomyzon*; *Erimyzon* and *Minytrema* are *incertae sedis* within Catostominae; and resurrection of the tribe Thoburniini, containing *Thoburnia* and expanded to include *Hypentelium*. © 2001 Academic Press

Key Words: Catostomidae; Cypriniformes; SSU mitochondrial DNA; LSU mitochondrial DNA; 12S mitochondrial DNA; 16S mitochondrial DNA; molecular phylogeny.

INTRODUCTION

Suckers (Catostomidae) are holarctic in distribution, being found across North America south in Central America to Guatemala; one species (*Catostomus catostomus*) is found in eastern Siberia and the genus *Myxocyprinus* is endemic to China (Berra, 1981; Smith, 1992). The family includes 76 recent species in 14 genera, with 13 genera and 75 species occurring in North and Central America (Table 1; Burr and Mayden, 1992; Jenkins and Burkhead, 1993). These 75 species constitute 7% of the North American ichthyofauna, with only minnows (Cyprinidae) and darters (Percidae) having more species. Even though this family constitutes a significant component of many aquatic ecosystems in North America, most systematic effort has been either alpha- (reviewed in Smith, 1992) or limited beta-level studies targeting the two largest tribes, Catostomini and Moxostomatini (Buth, 1978, 1979a,b, 1980; Jenkins, 1970; Robins and Raney, 1956, 1957; Smith, 1966; Smith and Koehn, 1971; although see Smith, 1992 discussed below). While not as speciose as either minnows or darters, catostomids display great diversity in endemism (Lee *et al.*, 1981), morphologies (Smith, 1966, 1992), gene evolution (e.g., Buth, 1978, 1979a; Ferris, 1984; Ferris and Whitt, 1980), and life history traits (e.g., Fuiman, 1985; Muth, 1990), all of which makes this group of great importance to studies of evolutionary biology. For example, species of suckers in North America display intriguing biogeographic patterns consistent with those of other fresh-

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TABLE 1

Classification of the Catostomidae proposed by Smith (1992), Following the Listing Convention of Nelson (1972, 1974)

Family Catostomidae
Subfamily Ictiobinae
Genera <i>Carpiodes</i> , <i>Ictiobus</i>
Subfamily Cycleptinae
Genera <i>Cycleptus</i> , <i>Myxocyprinus</i>
Subfamily Catostominae
Tribe Catostomini
Genera <i>Catostomus</i> , <i>Chasmistes</i> , <i>Deltistes</i> , <i>Xyrauchen</i>
Tribe Moxostomatini
Genera <i>Erimyzon</i> , <i>Hypentelium</i> , <i>Minytrema</i> , <i>Moxostoma</i> , <i>Scartomyzon</i> , <i>Thoburnia</i>

water fishes (Wiley and Mayden, 1985). The currently recognized Cycleptinae (*Myxocyprinus asiaticus* in China and *Cycleptus* in eastern North America) has an enigmatic east Asia-southeast North America biogeographic track replicated by some plants (Graham, 1972; Li, 1972; Wu *et al.*, 2000; Xiang *et al.*, 1998) and animals (e.g., paddlefishes, *Polyodon* and *Psephurus*, Grande and Bermis, 1991; giant salamanders, *Cryptobranchus* plus *Andrias* clade, and two species of *Alligator*, Burr and Mayden, 1999; and unionid mussels, Ortmann, 1912). Finally, catostomids are tetraploids

but display an amazing pattern of gene silencing or diploidization in their evolution (Buth, 1979a, 1982; Ferris, 1984; Ferris and Whitt, 1977, 1978, 1979). To effectively examine the evolution of these distributions, possible modes of speciation, and evolution of such traits, however, a well-corroborated phylogeny documenting ancestor/descendant relationships is required (Brooks and McLennan, 1991).

As stated above, most systematic efforts on catostomids prior to 1900 dealt with original descriptions and various contributions to higher-level classifications (reviewed by Smith, 1992). Subsequent contributions to catostomid classification include Hubbs (1930), who provided a key to eastern North American genera and designated tribes for these genera, and Robins and Raney's (1956) study of the genera and subgenera of *Moxostoma*. Nelson (1948, 1949) examined the Weberian apparatus and opercular series in catostomids; he concluded that these structures provided support for the subfamilial and tribal designations in Hubbs (1930). Miller (1959) depicted a phylogeny of the Catostomidae (Fig. 1A), which was based largely on Hubbs (1930) and Nelson (1948, 1949). In this discussion on relationships, Miller (1959, p. 199) suggested that the Cycleptinae might be divided into two subfamilies consistent with the disjunct distribution of *Cycleptus* (North America) and *Myxocyprinus* (China).

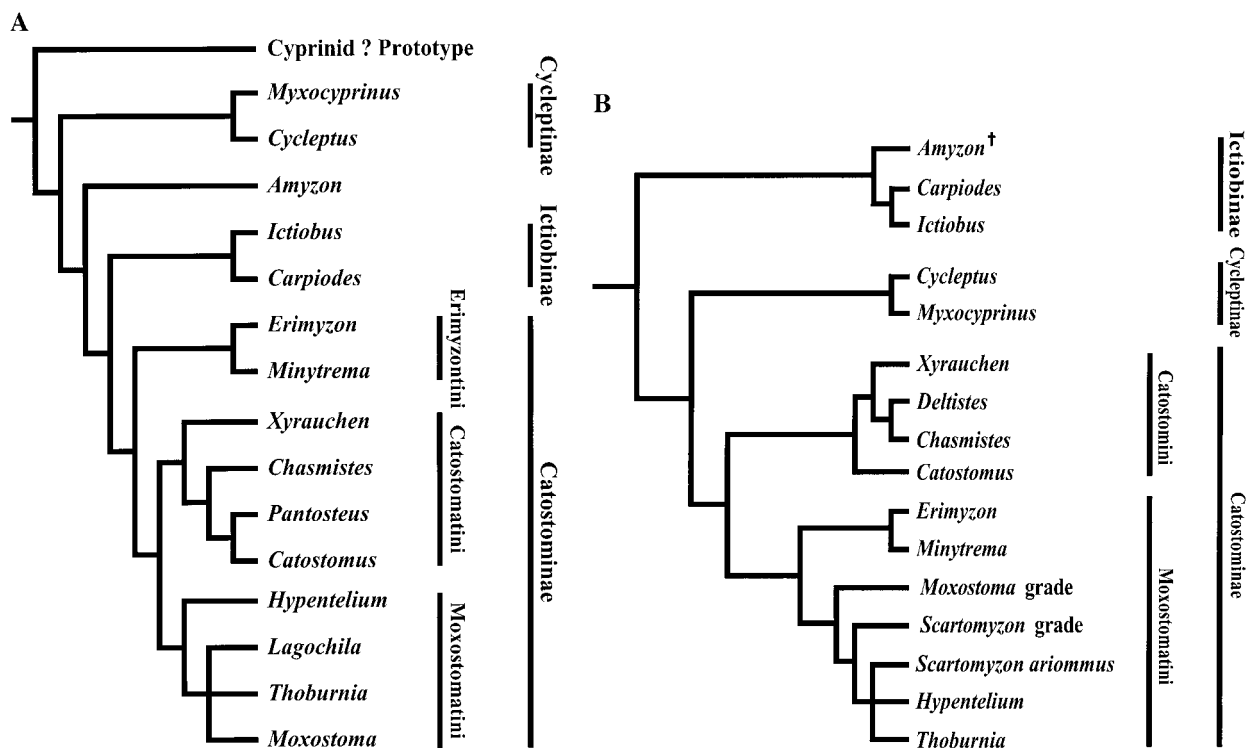


FIG. 1. Phylogenetic hypotheses of Catostomidae. (A) Miller's (1959) pre-Hennigian phylogeny, modified by placing taxonomic rankings alongside, rather than on, the tree. (B) Simplification of Smith's (1992) phylogenetic hypotheses based on morphological, biochemical, and early life history characters.

Ferris and Whitt (1978) constructed a phylogeny of 30 species based on the loss of duplicate gene expression in isozymes. Their Wagner tree placed the Ictiobinae as sister to Cycleptinae plus Catostominae. Within the Catostominae, they recognized three tribes, Erimyzonini, Moxostomatini, and Catostomini; *Moxostoma* was paraphyletic with *M. duquesnei* sister to *C. plebeius*, *C. platyrhynchus*, and *C. discobolus*.

Fuiman (1985) constructed a phylogeny of 17 species based on early life history characters. His phylogeny yielded a paraphyletic Ictiobinae basal to *Cycleptus elongatus* plus a monophyletic Catostominae, with the exception of *Erimyzon* which was part of an *Ictiobus* polytomy. Relationships among genera were not well resolved by developmental characters, with both *Catostomus* and *Moxostoma* being paraphyletic.

Smith (1992; Smith hereafter) provided the first comprehensive analysis of catostomid relationships based on 64 taxa and 157 morphological, biochemical, and early life history transformation series. Smith used this hypothesis to revise catostomid taxonomy and infer some modes of speciation and patterns of biogeography. Smith's analysis produced two equally parsimonious trees of 852 steps (CI = 0.35). In his preferred tree (Fig. 1B) the Ictiobinae was sister to Cycleptinae plus Catostominae, although Smith recognized that a limited number of characters supported this relationship; he also recognized that the possibility existed for an Ictiobinae plus Catostominae relationship based on a few homoplasious characters (characters not discussed). Within the Catostominae, Smith recognized two tribes, the Catostomini and Moxostomatini, because such a classification reflected the fundamental differences in characters among these taxa. Within the Moxostomatini, Smith's analysis yielded a paraphyletic *Moxostoma* grade sister to a paraphyletic *Scartomyzon* grade, which, in turn, was sister to an unresolved trichotomy of *Scartomyzon ariommus*, *Thoburnia*, and *Hypentelium*. The second topology produced by this analysis had *S. cervinus* as sister to an unresolved trichotomy of *S. ariommus*, *Thoburnia*, and *Hypentelium*.

Herein, we examine relationships among basal lineages of catostomids, employing mitochondrial (mt) DNA SSU and LSU rRNA gene sequences. Based on phylogenies resulting from analyses of the rRNA gene sequences, we propose a new classification for the Catostomidae that better reflects genealogical affinities within this family.

MATERIALS AND METHODS

Specimens Examined

We sequenced the entire mt rRNA SSU, intervening Valine tRNA, and LSU in 16 species of catostomids. These taxa represent all subfamilies and major clades

within subfamilies identified by Smith (1992; Fig. 1B). *Cyprinus carpio* (GenBank Accession No. X61010; Chang *et al.*, 1994) and *Carassius auratus* (GenBank Accession No. NC002079; Murakami *et al.*, 1998) were used as outgroups following Siebert (1987). Additional taxa included as functional outgroups representing the Cobitoidea (sensu Siebert, 1987) were *Crossostoma lacustre* (GenBank Accession No. NC_001727), *Botia macracantha* (local pet store, Tuscaloosa Co., AL; UAIC 12928.01), *Misgurnus anguillicaudatus* (local pet store, Tuscaloosa Co., AL; UAIC 12928.02), and *Gyrinocheilus aymonieri* (local pet store, Tuscaloosa Co., AL; UAIC 12928.03).

We examined the following species of Catostomidae: *Carpiodes carpio* (Honey Creek, Richardson Co., NE; UAIC 11219.08), *Catostomus catostomus* (Belt Creek, Cascade Co., MO; UAIC 11237.04), *C. commersoni* (Little Piney Creek, Phelps Co., MO; UAIC 11156.03), *Cycleptus elongatus* (Big Sunflower River, Big Sunflower Co., MS; UAIC 11371.01), *Erimyzon oblongus* (Mill Branch Creek, Colbert Co., AL; UAIC 11109.09), *Hypentelium nigricans* (Bear Creek, Marion Co., AL; UAIC 11138.02), *Ictiobus bubalus* (Paint Rock River, Marshall Co., AL; UAIC 11005.04), *Minytrema melanops* (Little Bear Creek, Franklin Co., AL; UAIC 11141.01), *Moxostoma anisurum* (Elk River, Limestone Co., AL; UAIC 11606.02), *M. carinatum* (Paint Rock River, Marshall Co., AL; UAIC 11005.03), *M. poecilurum* (Tallapoosa River, Tallapoosa Co., AL; UAIC 11442.01z), *Myxocyprinus asiaticus* (local pet store, Tuscaloosa Co., AL; UAIC 11698.01), *Scartomyzon ariommus* (South Fork Roanoke River, Montgomery Co., VA; UAIC 12071.01), *S. cervinus* (Craig Creek, Craig Co., VA; UAIC 11004.01), *Thoburnia rhothoeca* (Catawba Creek at Highway 31, Roanoke Co., VA; UAIC 11009.05), and *Xyrauchen texanus* (Lake Mohave, AZ; fin clip provided by D. Buth, UCLA). These taxa were selected to represent all subfamilies and major lineages within subfamilies or tribes as depicted in Smith (1992, Fig. 6).

DNA Amplification and Sequencing

The QIAGEN Qiampl tissue kit (Catalog No. 29304) was used to extract genomic DNA. Double-stranded DNA was amplified in overlapping fragments by polymerase chain reaction (PCR; Saiki *et al.*, 1988) with the primers listed in Table 2. Approximately 100 ng of genomic DNA was used as template for 25- μ l reactions containing 0.1 mM each dNTP, 10 μ M each primer, 2.5 μ l 10 \times *Taq* buffer (670 μ l 1 M Tris, 67 μ l 1 M MgCl₂, 83 μ l 2 M (NH₄)₂SO₄, 7 μ l 2-mercaptoethanol, 73 μ l ddH₂O, 100 μ l glycerol), and 1.25 units of *AmpliTaq* polymerase. Reactions were amplified for 35 cycles at 92°C for 60 s, 52°C for 60 s, and 72°C for 90 s. Double-stranded PCR products were sequenced on either an

TABLE 2

Primers Used in the Amplification and Sequencing of the 12S and 16S Genes for Catostomidae

Name	Sequence (5' → 3')	Strand	Reference
Phea	AAAGCACAGCACTGAAGATG	L	Titus and Frost (1996)
12Sa	AAGTGGGATTAGATACCCCACTA	L	Kocher <i>et al.</i> (1989)
12Sb	AGGAGGGTGACGGGCGGTGTGT	H	Simons and Mayden (1998)
12S414	ATCTAATCCCAGTTTGTCTCTCA	H	This study
12S897	AAGGGGAGGCAAGTCGTAACA	L	This study
16Sb	TTACCCTAGGGATAACAGCG	L	Simons and Mayden (1998)
16Sd	CAAGAGGCGATGTTTTT	H	Simons and Mayden (1998)
16Sd	AAAAACATCGCCTCCTG	L	Simons and Mayden (1998)
16Si	GAGTGGATAGAAGTTCAGCCT	L	Simons and Mayden (1998)
16S2086	AAGCCCTCGTTTAGCCATTCA	H	This study
16Sh	GCATAATAATCTAGCCAG	L	Simons and Mayden (1998)
LEU25	AGGGCTTAGGCCTTTCGCAA	H	Simons and Mayden (1998)

Applied Biosystems Inc. 377 or a 3100 Automated DNA Sequencer with a *Taq* DyeDeoxy Terminator Cycle Sequencing Kit.

Sequence Alignment and Analysis

All sequences were stored and initially aligned with XESEE (Cabot and Beckenbach, 1989). Proper sequence alignment is fundamental to any phylogenetic analysis of molecular data. This issue is particularly relevant to rRNA sequences, which have a complex secondary structure composed of short and long helices, loops, and bulges. Several recent studies have demonstrated that incorporation of secondary structure information greatly improved alignment and analyses of rRNA sequence data (e.g., Hickson *et al.*, 1996; Lydeard *et al.*, 2000). Alignment of catostomid mtDNA SSU sequences was refined with the *C. carpio* secondary structure model of Van de Peer *et al.* (1994); alignment of LSU sequences was refined with the *Bos taurus* secondary structure model based on sequences by Hauswirth and Laipis (1982; available from <http://www.rna.icmb.utexas.edu/RNA/23S/mitochondria.html>). Secondary structure information was manually incorporated with the alignment editor DCSE (De Rijk and De Wachter, 1993; available from <http://belgarath.uia.ac.be/dcse/>). The aligned data set including secondary structure information is available on request from the authors. All sequences are deposited in GenBank (Accession Nos. AF333592–AF333606 and AF357583–AF357586).

Hickson *et al.* (1996) demonstrated that use of traditional “stems” and “loops” categories in rRNA sequences was unsuitable because of differences in patterns of variation and conservation among rRNA structural classes. We identified the following five structural classes (Fig. 2) within the SSU and LSU sequence data based on the definitions of Hickson *et al.* (1996): (1) short helices—the two arms of the helix are close together in the primary structure of the DNA (Hixson and Brown, 1986); (2) long helices—the two

arms of the helix are separated by other helices in the primary structure of the DNA; (3) unpaired bases—nucleotides not within helices; (4) bulges—unpaired nucleotides within a helix; and (5) loops—“unpaired nucleotides within a helix (an internal loop) or between the proximal and distal arms of a helix (a hairpin loop)” (Hickson *et al.*, 1996, p. 152).

Pairwise comparisons of all taxa were generated by PAUP* (version 4.0b4a; Swofford, 1998). Comparisons of absolute numbers of transitions and transversions and transition:transversion ratios for each of the five rRNA structural classes were plotted against uncorrected genetic distance (p distance) for the SSU + Valine tRNA genes, the LSU gene, and all genes combined. Nucleotide variation and substitution patterns, including χ^2 test of homogeneity of base frequencies across taxa, were examined by PAUP*.

Phylogenetic Analysis

Phylogenies were estimated by maximum-parsimony analysis with the heuristic search option of PAUP* (100 random addition replications with tree bisection–reconstruction). Jackknife analysis with 1000 iterations was employed as a measure of internal stability of the data; “JAC” emulation and 37% data deletion per replicate were selected. The theoretical basis for these choices and a discussion on the statistical foundations of jackknifing and bootstrapping are presented in Farris *et al.* (1996). As an additional measure of tree stability, Bremer decay indices (Bremer, 1988, 1994) were calculated by TreeRot (Sorenson, 1996). A partition homogeneity test was conducted to determine the extent of conflict between SSU + Valine and LSU sequences with the incongruence length difference test (Farris *et al.*, 1995) as implemented in PAUP* (10 random addition replications, tree bisection–reconstruction, heuristic search option with 100 replications).

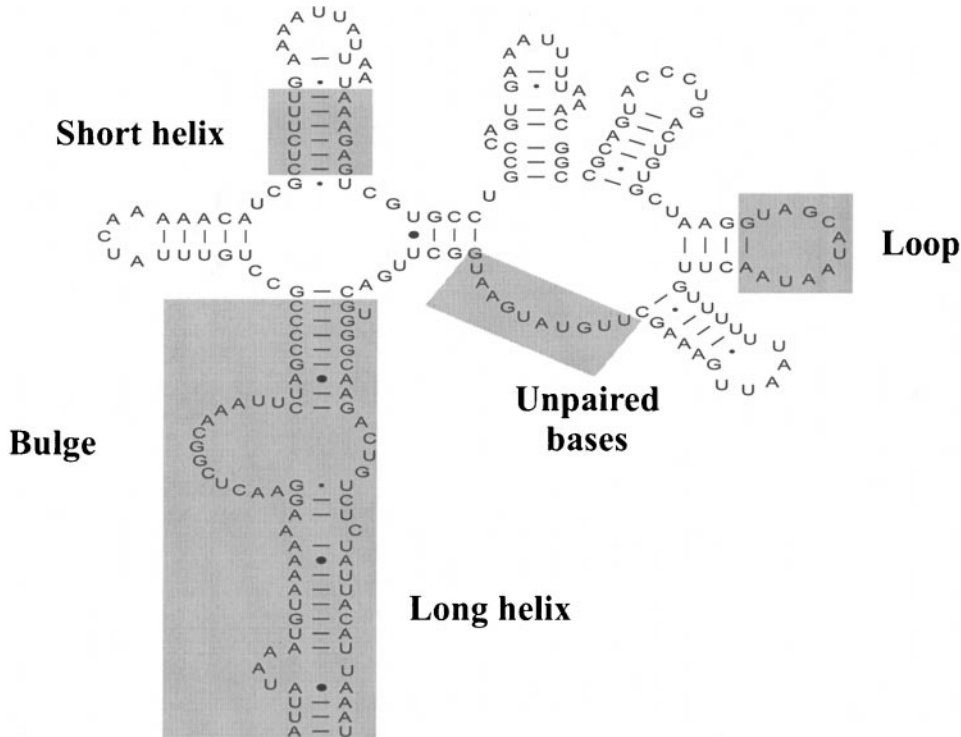


FIG. 2. Schematic representation of rRNA secondary structure.

RESULTS

Sequence Variation

Length of the mt rRNA SSU + Valine tRNA (collectively referred to as SSU hereafter) in catostomids was between 1021 and 1024 nt; LSU sequences ranged from 1674 to 1686 nt. Total sequence length (including gaps) was 2769 nt per specimen following alignment, for a complete data set (including outgroups) of 60,918 nt. No regions of ambiguous alignment were found in the SSU sequences; six regions of ambiguous alignment were found in the LSU sequences (positions 1472–1476, 1492–1495, 1503–1505, 1709–1714, 1891–1898, and 2748–2752; sequence position based on combined SSU and LSU sequence data) and were excluded from further analyses. There were 317 variable sites within the SSU sequence data, with 209 being potentially phylogenetically informative sites. Excluding ambiguous alignment sites, the LSU sequence data had 654 variable sites, 451 of which were potentially phylogenetically informative. The combined data set had 660 potentially phylogenetically informative sites. Table 3 shows the calculated pairwise genetic distances.

Pairwise comparisons of absolute numbers of transitions and transversions versus uncorrected genetic distance (p distance) were plotted for the five rRNA structural classes for the SSU, LSU, and combined sequence data. Patterns of variation were identical among the SSU, LSU, and combined sequence data sets, with only

unpaired bases and loop regions showing potential saturation of transitions relative to transversions (Figs. 3A–3E). We did not delete these structural classes from our analyses, however, because approximately 70% of potentially phylogenetically informative sites in the combined data set were found in either unpaired bases (254 sites) or loops (206 sites) and because of recent empirical justifications for not excluding potentially saturated sites from phylogenetic analyses (Broughton *et al.*, 2000). Because saturation can potentially affect phylogenetic analyses (e.g., Lydeard and Roe, 1997), differential weighting of transversions relative to transitions in the unpaired bases and loop regions was employed. Because it is difficult to make *a priori* decisions regarding weighting schemes, and such weighting may not be entirely justified (Broughton *et al.*, 2000), we limited our analyses to weighting transversions 1:1 and 2:1 (average transition:transversion ratio for unpaired bases plus loop regions for ingroup taxa was 1.79). In addition, short and long helices were downweighted 20% to compensate for helices sustaining a relatively greater number of compensatory mutations than is found in the other rRNA structural classes (Dixon and Hillis, 1993). Average nucleotide base frequencies for the SSU sequences were A = 0.31, C = 0.26, G = 0.23, and T = 0.20; for the LSU, A = 0.35, C = 0.23, G = 0.22, and T = 0.20. No differences in nucleotide base composition were observed among

TABLE 3
Pairwise Genetic Distances Based on Kimura's (1980) Two-Parameter Model

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. <i>Cyprinus</i>																	
2. <i>Carassius</i>	4.60																
3. <i>Myxocyprinus</i>	13.78	14.06															
4. <i>Carpoides</i>	14.20	13.21	8.94														
5. <i>Ictiobus</i>	14.27	14.25	8.19	5.42													
6. <i>Cycleptus</i>	14.14	14.80	9.23	7.61	6.61												
7. <i>C. catostomus</i>	14.39	14.24	9.32	8.87	8.26	8.40											
8. <i>C. commersoni</i>	12.86	13.49	8.86	7.79	7.13	7.00	2.75										
9. <i>Xyrauchen</i>	13.90	14.70	9.88	8.73	8.26	8.12	3.62	2.19									
10. <i>Erimyzon</i>	15.53	14.91	11.08	9.84	9.71	9.84	7.72	8.07	8.54								
11. <i>Minytrema</i>	13.75	13.91	9.43	8.44	8.04	8.64	5.33	5.34	6.05	7.45							
12. <i>M. anisurum</i>	14.27	14.68	10.65	9.07	9.09	9.01	7.59	7.01	7.47	8.67	6.45						
13. <i>M. carinatum</i>	14.76	15.07	11.01	9.48	9.30	9.02	7.80	7.21	7.68	9.03	6.38	3.37					
14. <i>M. poecilurum</i>	14.86	15.02	12.09	9.95	10.54	10.26	8.75	8.57	8.83	9.50	7.51	4.58	4.57				
15. <i>S. cervinus</i>	14.51	14.69	11.74	9.91	9.59	9.51	8.20	7.81	8.42	9.03	7.26	3.43	3.62	4.38			
16. <i>S. ariommus</i>	15.28	15.53	11.74	9.97	9.51	9.57	8.75	7.88	8.49	9.38	7.60	2.93	3.81	4.77	4.00		
17. <i>Thoburnia</i>	13.93	13.95	10.50	5.59	8.81	9.07	7.32	7.00	6.99	7.87	6.72	5.29	5.42	6.12	5.87	6.27	
18. <i>Hypentelium</i>	14.50	13.99	10.57	8.78	8.32	9.20	6.92	6.87	7.53	8.41	6.51	5.42	5.34	6.32	5.47	5.81	3.94

Note. Values are percentages.

taxa (SSU + Valine, $\chi^2 = 7.69$, $df = 63$, $P = 1.0$; LSU, $\chi^2 = 34.59$, $df = 63$, $P = 0.99$).

Phylogenetic Analyses

The partition homogeneity test indicated that the sequence data from the two genes could be combined ($P = 0.13$). Maximum-parsimony analysis employing different character weighting schemes on the three data sets yielded two basic topologies that differed only in resolution of the Cycleptinae (sensu Smith, 1992, Table 1), as either paraphyletic or monophyletic, and in relationships among some terminal taxa. These topologies and the differences in relationships among terminal taxa are discussed below.

All analyses yielded a monophyletic Cobitoidea, supported in 100% of jackknife replicates (Fig. 4). Within Cobitoidea, Cobitidae were sister to a clade of Gyrinocheilidae plus Catostomidae. In analyses of the combined and LSU data sets, this relationship was supported by 76–96% of jackknife replicates; jackknife support for this clade was <50% in analyses of the SSU data set.

In all analyses, Catostomidae were resolved as monophyletic, supported in 100% of jackknife replicates (Figs. 5 and 6, Table 4). Ictiobinae were monophyletic in all trees and supported in 68–98% of jackknife replicates. Combined and LSU analyses yielded a polyphyletic Cycleptinae; SSU analyses yielded a monophyletic Cycleptinae, but jackknife values were <50% except when helices were down-weighted 20% (52%; Table 4). SSU analyses also produced a Cycleptinae plus Ictiobinae clade, supported in 70–76% of jackknife replicates (Fig. 6). Catostominae also were resolved as monophyletic; jackknife support for this

clade was 67–88%. Within the Catostominae, the Catostomini were resolved as monophyletic, supported in 96–100% of jackknife replicates; however, *Catostomus* was never supported as monophyletic relative to *Xyrauchen*. The Moxostomatini were resolved as monophyletic in all analyses of the combined data set and LSU 2:1 analysis; jackknife support for this clade, however, was <50% except for the combined data set with helices down-weighted 20% (Table 4). The Moxostomatini were resolved as either para- or polyphyletic in the LSU 1:1 weighting, when helices were down-weighted 20%, and in all SSU analyses. Relationships within Moxostomatini, with the exception of *Erimyzon* and *Minytrema*, consistently identified *Thoburnia* and *Hypentelium* as either a basal paraphyletic grouping relative to *Moxostoma* plus *Scartomyzon* (all SSU analyses) or a monophyletic clade sister to the latter group (combined and LSU analyses). Jackknife values supporting the monophyly of *Thoburnia* and *Hypentelium* ranged from 92 to 100%. *Scartomyzon* was never found to be monophyletic, but to be a polyphyletic group always resolved within *Moxostoma*, rendering the latter paraphyletic.

All analyses of the combined data set yielded a single topology (combined 1:1 analysis: three trees of 2390 steps, CI = 0.560, RC = 0.312; Fig. 5) that varied only in the placement of *Erimyzon* and *Minytrema*. Jackknife support for basal nodes was 64–100%, with the exception of the *Cycleptus* plus Catostominae node, which ranged from 53 to 59% (<50% in the 2:1 analysis). Extension of this analysis to include trees of 2392 steps yielded seven additional trees. In these trees, the Cycleptinae (sensu Smith, 1992) were either a

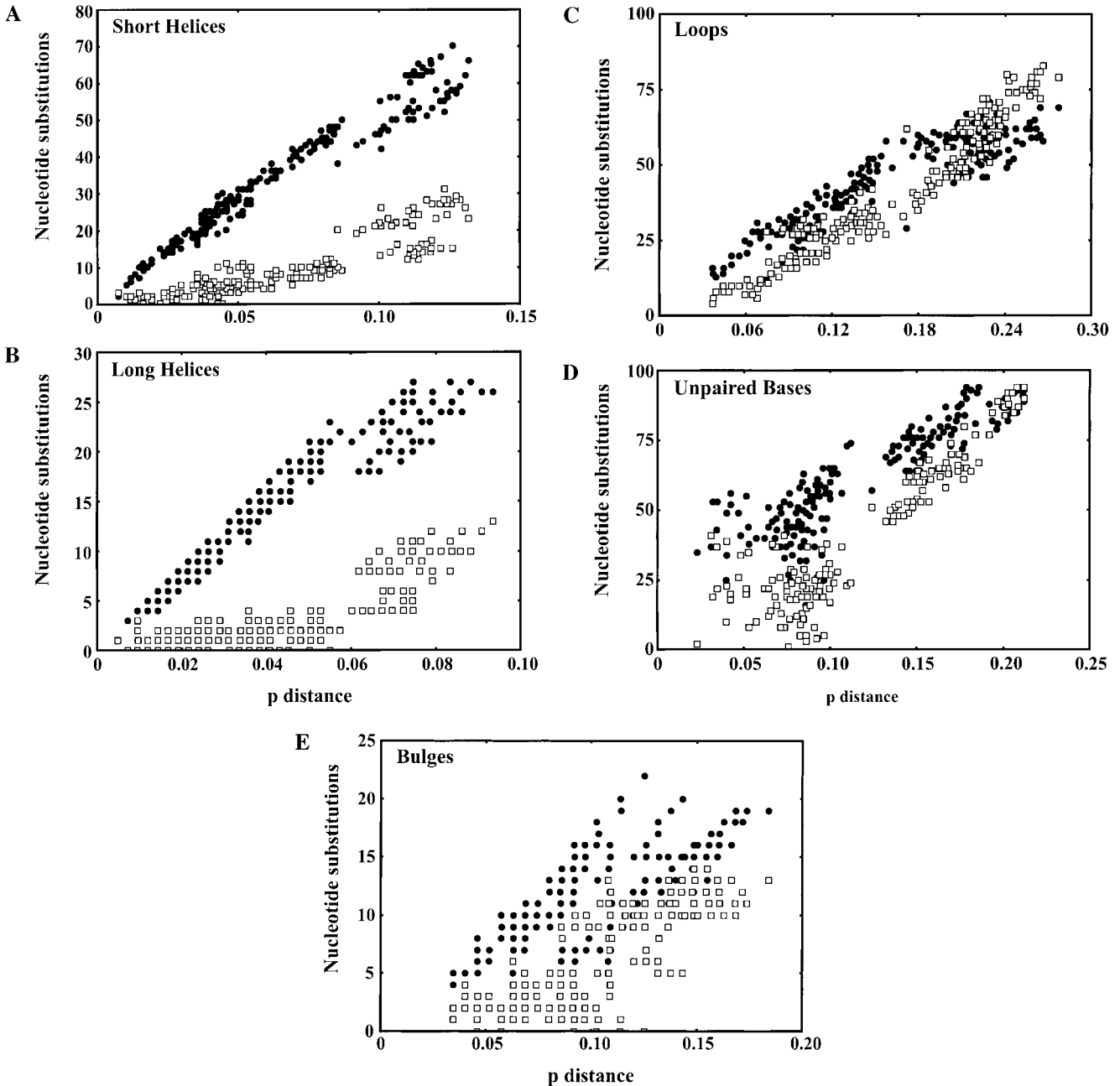


FIG. 3. Pairwise sequence comparison scatterplots showing absolute number of transitions and transversions against percentage sequence difference (p distance; uncorrected for multiple hits) for the five structural classes of rRNA. (A) short helices; (B) long helices; (C) loops; (D) unpaired bases; (E) bulges. Transitions, solid circles; transversions, open boxes.

paraphyletic group basal to Ictiobinae plus Catostominae (two trees) or a polyphyletic group as depicted in Fig. 5 (five trees). *Erimyzon* and *Minytrema* were basal to or in a trichotomy with *Thoburnia*, *Hypentelium*, *Moxostoma*, and *Scartomyzon*. Only the 2:1 analysis resolved *Erimyzon* and *Minytrema* as a monophyletic clade, supported in 62% of jackknife replicates and sister to remaining Moxostomatini.

Phylogenetic analyses of the LSU data set yielded

the same topology as that of the combined data set, with the exception of relationships within the Catostominae in the 1:1 and helices down-weighted 20% analyses (1:1 analysis: four trees of 1678 steps, CI = 0.557, RC = 0.301). In these two analyses, *Erimyzon* and *Minytrema* had varied positions, either singly or together, being sister to the Catostomini or the Moxostomatini or being basal to both tribes.

Analysis of the SSU sequence data with the three

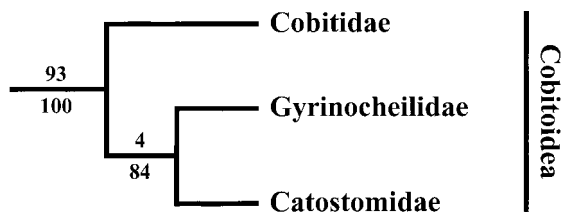


FIG. 4. Phylogenetic relationships of Cobitoidea based on parsimony analysis of the combined data set. Topology depicted is a simplified representation of the strict consensus tree yielded by the combined 1:1 weighting analysis (2390 steps; CI = 0.56; RC = 0.312). Numbers above branches are Bremer decay values; numbers below branches are jackknife replicates.

weighting schemes yielded two topologies, which again differed only in the placement of *Erimyzon* and *Minytrema*. As discussed above, both topologies yielded a monophyletic Cycleptinae with jackknife values $\leq 52\%$ (Fig. 6 and Table 4). These analyses also recovered a Cycleptinae plus Ictiobinae clade, supported in 70–76% of jackknife replicates. Within the Catostominae, *Erimyzon* and *Minytrema* were always recovered as a monophyletic group (jackknife support 52–60%), but varied in their relationship to remaining Catostominae. The 2:1 analysis yielded a single tree of 859 steps (CI = 0.591, RC = 0.365). In this tree, *Erimyzon* plus *Minytrema* was basal to the Catostomini plus remaining Moxostomatini, supported in 74% jackknife replicates. In 1:1 and helices down-weighted 20% analyses, the *Erimyzon* plus *Minytrema* clade was sister to either the Catostomini or the remaining Moxostomatini.

DISCUSSION

Phylogenetic analyses of the SSU, LSU, and combined data sets consistently resolved a monophyletic Cobitoidea (sensu Siebert, 1987), with Cobitidae sister to Gyrinocheilidae plus Catostomidae. These relationships differ from those derived by Siebert (1987), who placed gyrinocheilids basal to cobitids plus catostomids, but supports the hypothesis of Wu *et al.* (1981) based on morphological characters (although no synapomorphies for a Gyrinocheilidae plus Catostomidae clade were given) and is consistent with the suggestion of Rainboth *et al.* (1986).

Phylogenetic analyses of Catostomidae yielded two basic topologies with the weighting schemes employed; primary differences in the topologies were associated with the placement of *Myxocyprinus* and *Cycleptus* and relationships of *Erimyzon* and *Minytrema* to remaining Catostominae. Such different topologies may result from differing levels of phylogenetic signal among data sets (Avice, 1994; Ball *et al.*, 1990; Hillis and Huelsenbeck, 1992; Hillis, 1991). This is a likely possibility in our data given that there is an approximately 2 to 1 discrepancy in number of phylogenetically informative

sites between the two genes (SSU = 209 sites vs LSU = 451 sites). This situation is similar to that found by Simons and Mayden (1998) in their analysis of western North America minnows, in which LSU sequences contained three times as many phylogenetically informative sites as SSU sequences. Given that the partition homogeneity test did not indicate that separation of the data sets was warranted, we feel that simultaneous analyses of all available molecular data is the best approach to phylogenetic reconstruction when dealing with multiple topologies produced by different genes.

Figure 5 represents our preferred hypothesis of relationships within Catostomidae. This choice is based on the principle of total evidence analysis (sensu Kluge, 1989), the recovery of this topology in all analyses of the combined and LSU data sets, and the higher levels of jackknife support for basal nodes. In addition, this topology exhibits some congruence in both basal nodes and terminal taxa with aspects of both Miller's (1959) and Smith's (1992) phylogenetic hypotheses. Our molecular data consistently yielded a monophyletic Catostomidae, Ictiobinae, Catostominae, and Catostomini; the Moxostomatini were monophyletic in all combined and LSU 2:1 analyses, but were para- or polyphyletic in all remaining analyses. The Cycleptinae were paraphyletic in all combined and LSU analyses (Fig. 5), but were monophyletic in all SSU analyses (Fig. 6); in the combined and LSU analyses, *Myxocyprinus* was the basal-most taxon and *Cycleptus* was sister to Catostominae.

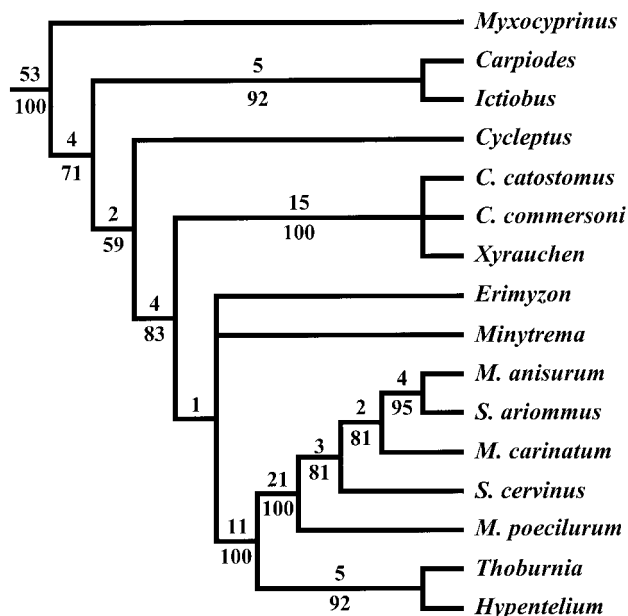


FIG. 5. Phylogenetic relationships of Catostomidae based on parsimony analyses of the combined and LSU data sets. Topology depicted is the strict consensus of three trees yielded by the combined 1:1 analysis (2390 steps; CI = 0.560; RC = 0.312). Numbers above branches are Bremer decay values; numbers below branches are jackknife replicates.

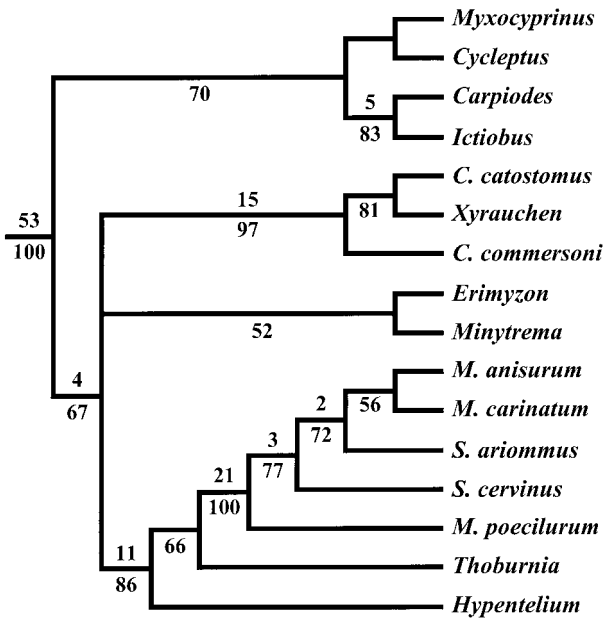


FIG. 6. Phylogenetic relationships of Catostomidae based on parsimony analyses of the SSU data set. Topology depicted is the strict consensus of three trees yielded by the SSU 1:1 analysis (699 steps; CI = 0.579; RC = 0.351). Numbers above branches are Bremer decay values; numbers below branches are jackknife replicates.

Paraphyly of the Cycleptinae is inconsistent with both Miller's (1959) and Smith's (1992) phylogenetic hypotheses of the Catostomidae. Such incongruence between molecular and morphological phylogenies results from factors such as (1) the molecular data do not provide useful phylogenetic information at a particular taxonomic level, resulting in an incorrect gene tree; (2) the morphological phylogenetic hypothesis might be incorrect; or (3) elements of both topologies might be incorrect due to equivocal data for those nodes (Lydeard and Roe, 1997). Smith (1992) listed 20 apomorphies supporting the Cycleptinae plus Catostominae relationship (his Table 2); 14 of 20 characters were ordered, multistate transformation series (TS), 4 were binary TS, and 2 were unordered, multistate TS. No unequivocal synapomorphies supporting a Cycleptinae plus Catostominae clade were identified. Given the debate that has occurred in the systematic literature over the use of ordered versus unordered transformation series in phylogenetic reconstruction (e.g., Donoghue and Maddison, 1986; Hauser and Presch, 1991; Mabee, 1989a,b, 1993), we used MacClade (Maddison and Maddison, 1992) to examine the support for this relationship with the TS ordered and unordered. Four ordered TS (Smith's characters 61, 96, 97, 113) had equivocal distributions not supporting this arrangement; an additional four characters (55, 133, 136, 152) were identified as synapomorphies for the node because of character ordering, but were equivocal in their support when unordered. Smith (1992, p. 797) con-

ceded that the Ictiobinae may be the sister group to Catostominae based on "a few interesting" homoplasious characters, but did not list these characters. When TS were treated as unordered, eight (2, 4, 21, 32, 33, 39, 65, 146) supported the sister group relationship of Ictiobinae plus Catostominae. Thus, the incongruence at this node may be due, in part, to ambiguous morphological character support for this node and phylogenetic analyses enforcing order on transformation series. Basal placement of the Cycleptinae is consistent with Miller's (1959) pre-Hennigian phylogeny of catostomids; the sister group relationship between *Cycleptus* plus Catostominae is consistent with Ferris and Whitt's (1978) phylogeny based on the loss of duplicate gene expression (with the caveat that *Myxocyprinus* was not examined in that study). An unnatural Cycleptinae is also consistent with recent biochemical data, which revealed that few allozyme loci were shared between *Myxocyprinus* and either *Cycleptus* or *Ictiobus* (Buth, 1998).

Our analyses of molecular data yielded a monophyletic Catostomini, consistent with Smith's (1992) analysis. All combined and LSU analyses yielded a trichotomy of *C. catostomus*, *C. commersoni*, and *Xyrauchen*; all SSU analyses depicted *Xyrauchen* as sister to either of the two species of *Catostomus*, rather than basal to a *Catostomus* clade. *Catostomus* was never recovered as monophyletic. Given that there is no Bremer decay support for this node in the SSU analyses, however, the potential paraphyletic nature of *Catostomus* is tenuous at best. The relationship between *Xyrauchen* and *Catostomus* may be real or could result from the conservative nature of both the SSU and the LSU genes, which probably have insufficient molecular variation for determination of relationships within the Catostomini and/or incomplete taxon sampling. Inclusion of *Chasmistes*, *Deltistes*, and other *Catostomus* species and examination of more rapidly evolving genes will help to clarify relationships within the Catostomini.

TABLE 4

Range of Jackknife Values $\geq 50\%$ Supporting Taxonomic Rankings (sensu Smith, 1992) from Analyses of the Combined, LSU, and SSU Data Sets

	Data set		
	Combined	LSU	SSU
Catostomidae	100 (3)	100 (3)	100 (3)
Cycleptinae			52 (1)
Ictiobinae	91-98 (3)	68-93 (3)	82-90 (3)
Catostominae	81-85 (3)	82-88 (3)	67-74 (3)
Catostomini	100 (3)	100 (3)	96-97 (3)
Moxostomatini	53 (1)		

Note. Numbers in parentheses are numbers of analyses (1:1, 2:1 weighting and helices downweighted 20%) for which jackknife values were reported.

In our analyses, phylogenetic affinities of *Erimyzon* and *Minytrema* varied depending upon the data set and weighting scheme; singly or together these two taxa were either sister to the Catostomini, sister to the Moxostomatini, or basal to a Catostomini plus remaining Moxostomatini clade. Placement of *Erimyzon* plus *Minytrema* as basal to a Catostomini plus Moxostomatini clade is consistent with Miller's (1959; Fig. 1A) pre-Hennigian hypothesis; placement of *Erimyzon* plus *Minytrema* as basal to a clade inclusive of *Thoburnia*, *Hypentelium*, *Moxostoma*, and "*Scartomyzon*" is consistent with Smith's (1992) hypothesis (Fig. 1B). Smith (1992) noted that recognition of *Erimyzon* plus *Minytrema* as the tribe Erimyzonini (sensu Hubbs, 1930) was consistent with his phylogenetic hypothesis, but he chose to include these taxa within his Moxostomatini to reflect the more fundamental differences between the Catostomini and the Moxostomatini. Given the uncertain phylogenetic affinities of *Erimyzon* and *Minytrema* within the subfamily Catostominae, we identify them as *incertae sedis* (Table 5).

In analyses of the combined, SSU, and LSU data sets, both species of *Scartomyzon* were resolved to be embedded within *Moxostoma*, questioning the monophyly of both *Moxostoma* and *Scartomyzon* if the latter genus is recognized as a distinct taxon. Both *Moxostoma* and *Scartomyzon* formed paraphyletic grades in Smith (1992), suggesting that some species currently recognized in these genera may be more closely related to other *Moxostoma* or a *Thoburnia* plus *Hypentelium* clade or they may form distinct evolutionary lineages. Jenkins (1970) suggested that *S. ariomus* was closely related to *T. atripinnis*; Smith (1992) placed *S. ariomus* in a trichotomy with *Thoburnia* plus *Hypentelium*. Analyses of the combined and LSU data sets consistently resolved an *S. ariomus* plus *M. anisurum* relationship, whereas analyses of the SSU data set also yielded a paraphyletic *Scartomyzon* grade. Constraining *Scartomyzon* to be monophyletic yielded one tree of 2399 (nine steps longer than the most parsimonious combined 1:1 tree); in this tree, *S. ariomus* plus *S. cervinus* were sister to *M. anisurum* and these three taxa were terminal within *Moxostoma*. Whereas additional taxa of *Scartomyzon* and *Moxostoma* are needed to expand these data sets and further elucidate the composition of, and limits to, both genera, it is clear that the genealogical affinities of *S. ariomus* and *S. cervinus* are within *Moxostoma* and that *Scartomyzon* is not a monophyletic group.

Analysis of the SSU data placed *Thoburnia* and *Hypentelium* as a clade basal to a grade of *Moxostoma* and *Scartomyzon*, supporting previous interpretations of relationships based on morphological and biochemical characters (Bailey, 1959; Buth, 1979a,b). In analyses of the combined and LSU data sets, however, *Thoburnia* and *Hypentelium* form a clade sister to the composite *Moxostoma* plus *Scartomyzon* clade (Fig. 5), which

TABLE 5

Classification of the Catostomidae Based on mtDNA SSU and LSU rRNA Gene Sequences

Family Catostomidae
Subfamily Myxocyprinae "New Subfamily"
Genus <i>Myxocyprinus</i> (China, Yangtse River).
Subfamily Ictiobinae
Plesion <i>Amyzon</i> † (North America)
Genus <i>Carpiodes</i> (Eastern and Central North America)
Genus <i>Ictiobus</i> (Eastern and Central North America, south to Guatemala)
Subfamily Cycleptinae
Genus <i>Cycleptus</i> (Eastern and Central North America)
Subfamily Catostominae
Genus <i>Erimyzon</i> (Eastern North America) <i>incertae sedis</i>
Genus <i>Minytrema</i> (Eastern North America) <i>incertae sedis</i>
Tribe Catostomini
Genus " <i>Catostomus</i> " (North America and Siberia)
Genus <i>Xyrauchen</i> (Western North America)
Genus <i>Chasmistes</i> ? (Western North America)
Genus <i>Deltistes</i> ? (Western North America)
Tribe Thoburniini (sensu Hubbs, 1930)
Genus <i>Thoburnia</i> (Eastern North America)
Genus <i>Hypentelium</i> (Eastern North America)
Tribe Moxostomatini
Genus " <i>Moxostoma</i> "
Genus " <i>Scartomyzon</i> "?

Note. Potentially para- or polyphyletic groups are noted in shutter quotes; groups that may not warrant recognition, or that have uncertain placement, are followed by a "?"

agrees with Smith (1992) and is consistent with Jenkins' (1970) pre-Hennigian phylogeny. Given the greater number of phylogenetically informative sites in the LSU data set and the high jackknife and Bremer decay values generated in the combined analyses, we argue that *Thoburnia* and *Hypentelium* form a clade sister to a monophyletic group inclusive of the composite "*Moxostoma*" and "*Scartomyzon*," rather than a paraphyletic grouping basal to these latter taxa. We suggest placing *Thoburnia* (which has an obsolete swim bladder in adults) and *Hypentelium* (which has a greatly reduced swim bladder) in the tribe Thoburniini and restricting Moxostomatini to "*Moxostoma*" and "*Scartomyzon*" (whose taxa have a three-chambered swim bladder). This classification more accurately reflects phylogenetic relationships among these taxa and better represents the tenets of Hennigian classification (Table 4).

Based on the phylogeny in our preferred tree (Fig. 5), we offer a classification of the Catostomidae that better reflects the genealogical relationships elucidated by our analyses (Table 5). In this classification, we employ the listing convention of Nelson (1972, 1974). We note potentially para- or polyphyletic groups in shutter quotes; groups that may not warrant recognition, or that have uncertain placement, are followed by a "?"

Whereas this study has advanced our knowledge of basal relationships among catostomids, there are

clearly several issues pertaining to relationships within the Catostominae that require further elucidation. For example, the phylogenetic affinities of *Erimyzon* and *Minytrema*, whether within the Moxostomatini or the Catostomini or basal to both of these tribes, needs to be resolved. Similarly, the composition and relationships of taxa presently identified as belonging to either "*Moxostoma*" or "*Scartomyzon*" needs further study, as do species relationships among genera within the Catostomini. Phylogenetic relationships among, and the taxonomic composition of, *Catostomus*, *Chasmistes*, *Deltistes*, and *Xyrauchen* are especially needed, given the endangered or threatened status of species within these genera.

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