Volume 54   Issue 3   March 2010   ISSN 1055-7903

ELSEVIER

MOLECULAR
PHYLOGENETICS
& EVOLUTION

EDITOR-IN-CHIEF:
MORRIS GOODMAN

Short Communication

Phylogenetic relationships of catostomid fishes (Actinopterygii: Cypriniformes) based on mitochondrial ND4/ND5 gene sequences

Michael H. Doosey [a,b,*], Henry L. Bart Jr. [a,b], Kenji Saitoh [c], Masaki Miya [d]

[a] Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA 70118, USA
[b] Tulane University Museum of Natural History, Belle Chasse, LA 70037, USA
[c] National Research Institute of Fisheries Science, Aquatic Genomics Research Center, Yokohama 236-8648, Japan
[d] Department of Zoology, Natural History Museum and Institute – Chiba, 955-2 Aoba-cho, Chuo-ku, Chiba 260-8682, Japan

ABSTRACT

Family Catostomidae is a diverse group of benthic freshwater fishes that are distributed across North America and in parts of East Asia. In this study, the phylogenetic relationships of Catostomidae is examined using 3436 nucleotides of mitochondrial ND4 and ND5 protein coding genes and intervening tRNAs. All 13 genera and 60 species of catostomids were sampled to represent diversity of the family. Catostomidae and its four subfamilies were found to be monophyletic; however, relationships of the subfamilies are not strongly supported with bootstrapping. The analysis provides strong support for recognizing four tribes in subfamily Catostominae.

© 2009 Elsevier Inc. All rights reserved.

1. Introduction

Family Catostomidae, commonly known as suckers, is a diverse group of primarily benthic fishes that inhabit temperate lotic and lacustrine habitats of North America and East Asia (Smith, 1992). The majority of species are distributed in Canada, United States, and Mexico. Only two species occur in Asia, one in China and one in Siberia (Smith, 1992). All catostomids are tetraploid (Uyeno and Smith, 1972) and the genome duplication event may have been important for diversification of the group. Catostomidae comprises 13 genera, 72 described species in four subfamilies: Myxocyprininae, Ictiobinae, Cycleptinae, and Catostominae (Nelson, 2006).

Catostomids have received much attention from taxonomists and systematists, who have used a diverse array of characters to establish classifications and/or hypotheses of relationships for the group. Hubbs (1930) used morphological characters to establish four tribes of subfamily Catostominae: Catostomini, Erimyzonini, Moxostomatini, and Thoburniini. Robins and Raney (1956) used morphology to described subgenera and species of Moxostoma. Smith (1966) and Smith and Koehn (1971) provided accounts of taxonomy, distribution, and evolution of species of Catostomus. Jenkins's (1970) unpublished Ph.D. thesis on Moxostomatini

included descriptions of subgenera and a hypothetical phylogeny. Ferris and Whitt (1978) utilized isozymes to characterize loss of duplicated gene expression in suckers, and generated a Wagner cladogram that depicted Ictiobinae as sister to Cycleptinae plus Catostominae. Buth (1979) proposed phenetic and phylogenetic relationships of Thoburnia based on isozyme variability. Fuiman (1985) employed larval characters to build a phylogeny of 17 catostomids.

Smith's (1992) comprehensive phylogeny involving 157 morphological, biochemical, and early life history characters supported a monophyletic Catostomidae and three monophyletic subfamilies, consistent with Ferris and Whitt (1978). Smith (1992) recognized two tribes, Catostomini and Moxostomatini, within Catostominae. Within Moxostomatini, Moxostoma was paraphyletic and sister to a paraphyletic Scartomyzon which was sister in turn to an unresolved group comprising Hypentelium, Thoburnia, and S. ariommus.

Harris and Mayden (2001) utilized 12S and 16S rRNA characters to resolve a monophyletic Catostomidae and four monophyletic subfamilies, including the new subfamily Myxocyprininae. Their preferred hypothesis of relationships was (Myxocyprininae, (Ictiobinae, (Cycleptinae, Catostominae))). In a phylogeny proposed by Harris et al. (2002) based on cytochrome b (cyt b) gene sequences, Catostominae was strongly supported, but there was no consistent support for Erimyzonini (Minytrema plus Erimyzon). Catostomus was not monophyletic because Deltistes and Xyrauchen were nested within the Catostomus clade. A large clade comprising Thoburnia, Hypentelium, Moxostoma, and Scartomyzon was consis-

* Corresponding author. Address: Tulane University, Department of Ecology and Evolutionary Biology, 400 Boggs Center, New Orleans, LA 70118, USA. Fax: +1 504 862 8706.
E-mail address: mdoosey@tulane.edu (M.H. Doosey).

tently recovered, but there was no consistent support for recognizing *Thoburnia* separate from *Hypentelium*, or *Scartomyzon* separate from *Moxostoma*.

Saitoh et al. (2006) sequenced the complete mtDNA genome for seven catostomid species and obtained the following relationships of subfamilies ((Myxocyprininae, (Ictiobinae, Cycleptinae)), Catostominae). Sun et al. (2007) proposed a peculiar UMPGA tree based on cyt *b* sequence data that resulted in a nonmonophyletic Catostominae which strongly contrasted Harris et al. (2002). Lastly, in a consensus phylogeny for order Cypriniformes based on four nuclear genes with limited sampling of catostomids, Mayden et al. (2009) recovered a basal Cycleptinae sister to Myxocyprininae plus Catostominae; Ictiobinae was not included in the study.

Two common problems likely contributed to the inconsistency of previous attempts to resolve relationships of catostomids based on DNA sequence data: limited taxon sampling and poor choices of genes for study. The mitochondrial ND4/ND5 gene region has been shown to outperform commonly utilized mtDNA genes such as CO1, cyt *b*, and 12S/16S rRNA genes in phylogenetic analysis at broad taxonomic scales because it is relatively long (ca. 3.4 kb) and contains more phylogenetically informative variation at first and second codon positions (Miya et al., 2006). Moreover, the ND4/ND5 gene region is easier to amplify and sequence for large numbers of taxa than the whole mitochondrial genome, especially when employing the primers and protocol suggested by Miya et al. (2006). We use sequence data from ND4/ND5 to infer phylogenetic relationships of 60 catostomid species, representing all genera, and compare the results to previous hypotheses of relationships.

2. Methods and materials

2.1. DNA extraction, PCR, and sequencing

DNA was extracted from ethanol preserved tissue with the Purelink Genomic DNA Mini Kit (Invitrogen). PCR amplification was conducted in two steps, long PCR and full-nested short PCR (Table 1) with a protocol modified from Miya et al. (2006). The long PCR primer pair L10474-Arg-C and H14710-Glu-C was used in a 15.5-μL reaction mixture containing 9.725 μL water, 1.5 μL 10× PCR buffer, 1.2 μL dNTP (2.5 mM), 1.0 μL each primer (10 μM), 0.075 μL polymerase (Takara *Ex Taq*), and 1.0 μL of template DNA (ca. 50 ng/μL). The six primers pairs suggested by Miya et al. (2006) were used to amplify overlapping short segments with

Table 1
Thermal cycling profile used to amplify ND4/ND5 gene region in two steps, long PCR (A) and full-nested short PCR (B). Catostomid specific primers used as needed to fill gaps in contigs (C).

Step	Temp. (°C)	Time (s)
A – long PCR		
Initial denaturation	95	120
Next 3 steps for 30 cycles		
Denaturation	94	30
Annealing	55	5
Extension	68	420
Final extension	72	300
B – short PCR		
Initial denaturation	95	120
Next 3 steps for 30 cycles		
Denaturation	94	30
Annealing	52	30
Extension	72	60
Final extension	72	300
C – catostomid specific PCR primers		
L11778-ND4C	5′-GCCTAYGARCGMACCCATAGCCG-3′	
L11892-ND4C	5′-CCMCTCCCAAACYTAATRGGAGA-3′	
H12531-ND5C	5′-TCAAATGTKGYRGTRTTTATTCA-3′	
H12571-ND5C	5′-GARTARTTGTCRAATTTRAAGCT-3′	

the same reaction mixture as for long PCR. Catostomid specific primers were designed and used as necessary to fill gaps (Table 1). Short PCR products were purified with diluted (1:19) ExoSAP-IT (USB) and directly sequenced using Big Dye 3.1 terminator cycle sequencing kit (Applied Biosystems) following the manufacturer's protocol then visualized on an ABI 3730*xl* automated DNA sequencer.

2.2. Sequence alignment and phylogenetic analysis

Sequence chromatograms were edited with Sequencher 4.6 (Gene Codes). Additional sequences were obtained from NCBI. Sequences were aligned manually and gaps inserted to improve alignment were treated as missing data. Intervening tRNA genes (His, Ser$_1$, and Leu$_2$) were aligned manually following the putative secondary structure shown in Kumazawa and Nishida (1993). Sequence divergence and patterns of sequence variation for each codon position of the protein coding genes were performed with PAUP* (Swofford, 2002). An alignment of cyt *b* sequences was created and sequence variation was examined to compare ND4/ND5 genes with Harris et al. (2002).

A maximum likelihood (ML) approach to phylogenetic reconstruction was employed. Two data sets (both including ND4/ND5 genes partitioned by codon and tRNAs) were constructed for Bayesian and RAxML analysis. One data set included all substitutions; the other utilized RY-coding for third position substitutions in ND4/ND5 genes. To produce the RY-coded data set, third position purines (R) were coded 'A' and third position pyrimidines (Y) were coded 'C', following Saitoh et al. (2006). Partitioned Bayesian phylogenetic analysis with 3×10^6 iterations of MCMC was performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) and trees were saved each 1000 generations. Additional ML estimation of the phylogenetic tree was performed with RAxML (Stamatakis et al., 2008) and bootstrapped 1000 times. Modeltest 3.06 (Posada and Crandall, 1998) identified GTR+I+Γ as most appropriate for codon partitions and HKY+I+Γ as most appropriate for the tRNA genes.

3. Results

3.1. Genetic difference and saturation

ND4/ND5 sequence data were collected for 60 catostomid species representing all extant genera (Table 2). Lengths of the ND4 and ND5 genes were 1383 and 1839 nt, respectively. The tRNA genes were each about 70 nt, making the total length of the concatenated sequence 3436 nt. The complete ND4/ND5 data set comprised 1381 (39.6%) parsimony informative sites, distributed among codon positions as follows: 287 first position (20.9%); 73 second position (5.3%); 1014 third position (73.8%). Mean Tamura-Nei distance was 0.18 with a range of 0.0034–0.26 for the species pairs of *Ictiobus niger/I. cyprinellus* and *Carpiodes velifer/Catostomus nebuliferus*, respectively. There was no evidence of saturation at first and second codon positions, but there appeared to be some saturation of third position transitions (Supplementary Fig. S1).

Order Cypriniformes was recovered as a monophyletic group with strong support. Cyprinidae was recovered as the most likely sister group of catostomids. This analysis suggests that Superfamily Cobitoidea is paraphyletic.

3.2. Interrelationships of Catostomidae

All analyses resolved a monophyletic Catostomidae and produced similar relationships of the subfamilies (Figs. 1 and 2).

Table 2
List of species used in this study including GenBank accession number and classification of the Catostomidae.

Classification	Species	Accession No.	Reference
Order Cypriniformes			
Family Catostomidae			
Subfamily Catostominae			
Tribe Erimyzonini	*Erimyzon oblongus*	AP011228	Miya unpublished
	Erimyzon sucetta	FJ751824	This study
	Minytrema melanops	AB242166	Saitoh et al. (2006)
Tribe Catostomini	*Catostomus ardens*	FJ751806	This study
	Catostomus bernardini	FJ751807	This study
	Catostomus catostomus catostomus	FJ751808	This study
	Catostomus clarkii	FJ751813	This study
	Catostomus columbianus	FJ751810	This study
	Catostomus commersonii	AB127394	Saitoh et al. (2006)
	Catostomus discobolus	FJ751811	This study
	Catostomus fumeiventris	FJ751812	This study
	Catostomus insignis	FJ751809	This study
	Catostomus latipinnis	FJ751814	This study
	Catostomus macrocheilus	FJ751815	This study
	Catostomus nebuliferus	FJ751816	This study
	Catostomus occidentalis	FJ751817	This study
	Catostomus plebeius	FJ751818	This study
	Catostomus rimiculus	FJ751819	This study
	Catostomus santaanae	FJ751820	This study
	Catostomus warnerensis	FJ751821	This study
	Chasmistes brevirostris	FJ751822	This study
	Deltistes luxatus	FJ751823	This study
	Xyrauchen texanus	EU265776	Unpublished
Tribe Thoburniini	*Hypentelium etowanum*	FJ751825	This study
	Hypentelium nigricans	AB242169	Saitoh et al. (2006)
	Hypentelium roanokense	FJ751826	This study
	Thoburnia atripinnis	FJ751850	This study
	Thoburnia hamiltoni	FJ751851	This study
	Thoburnia rhothoeca	FJ751852	This study
Tribe Moxostomatini	*Moxostoma albidum*	FJ751830	This study
	Moxostoma anisurum	FJ751831	This study
	Moxostoma ariommum	FJ751832	This study
	Moxostoma austrinum	FJ751833	This study
	Moxostoma breviceps	FJ751834	This study
	Moxostoma carinatum	FJ751835	This study
	Moxostoma cervinum	FJ751836	This study
	Moxostoma collapsum	FJ751837	This study
	Moxostoma congestum	AP009317	Saitoh unpublished
	Moxostoma duquesnii	FJ751838	This study
	Moxostoma erythrurum	FJ751839	This study
	Moxostoma hubbsi	FJ751840	This study
	Moxostoma lachneri	FJ751841	This study
	Moxostoma macrolepidotum	FJ751842	This study
	Moxostoma mascotae	FJ751843	This study
	Moxostoma pappillosum	FJ751844	This study
	Moxostoma poecilurum	AB242167	Saitoh et al. (2006)
	Moxostoma robustum	FJ751845	This study
	Moxostoma valenciennesi	FJ751849	This study
	Moxostoma sp. "brassy jumprock"	FJ751846	This study
	Moxostoma sp. "sickle fin redhorse"	FJ751847	This study
	Moxostoma sp. "Apalachicola redhorse"	FJ751848	This study
Subfamily Cycleptinae	*Cycleptus elongatus*	AB126082	Saitoh et al. (2006)
Subfamily Myxocyprininae	*Myxocyprinus asiaticus*	AP006764	Saitoh et al. (2006)
Subfamily Ictiobinae	*Carpiodes carpio*	AP006763	Saitoh et al. (2006)
	Carpiodes cyprinus	FJ751804	This study
	Carpiodes velifer	FJ751805	This study
	Ictiobus bubalus	AP009316	Saitoh unpublished
	Ictiobus cyprinellus	FJ751827	This study
	Ictiobus meridionalis	FJ751828	This study
	Ictiobus niger	FJ751829	This study
Outgroups			
Family Cyprinidae	*Alburnus alburnus*	AB239593	Saitoh et al. (2006)
	Aphyocypris chinensis	AB218688	Saitoh et al. (2006)
	Carassius auratus langsdorfii	AB006953	Murakami et al. (1998)
	Culter mongolicus	AP009060	Saitoh et al. (2006)
	Cyprinella lutrensis	AB070206	Saitoh et al. (2006)
	Cyprinus carpio	AP009047	Mabuchi et al. (2006)
	Gobio gobio	AB239596	Saitoh et al. (2006)
	Hemibarbus barbus	AB070241	Saitoh et al. (2006)
	Labeo batesii	AB238967	Saitoh et al. (2006)
Family Balitoridae	*Homaloptera leonardi*	AB242165	Saitoh et al. (2006)

Table 2 (continued)

Classification	Species	Accession No.	Reference
Family Cobitidae	*Acantopsis choirorhynchus*	AB242161	Saitoh et al. (2006)
	Cobitis striata	AB054125	Saitoh et al. (2003)
	Misgurnus nikolskyi	AB242171	Saitoh et al. (2006)
	Pangio anguillaris	AB242168	Saitoh et al. (2006)
Family Nemacheilidae	*Barbatula toni*	AB242162	Saitoh et al. (2006)
Family Gyrinocheilidae	*Gyrinocheilus aymonieri*	AB242164	Saitoh et al. (2006)
Order Siluriformes			
Family Bagridae	*Pseudogabrus tokiensis*	AB054127	Saitoh et al. (2003)
Family Callichthyidae	*Corydoras rabauti*	AB054128	Saitoh et al. (2003)
Order Characiformes			
Family Alestiidae	*Phenacogrammus interruptus*	AB054129	Saitoh et al. (2003)
Family Characidae	*Chalceus macrolepidotus*	AB054130	Saitoh et al. (2003)

Two clades of equal rank were resolved in best ML trees, one comprising a basal Cycleptinae, sister to Myxocyprininae plus Ictiobinae; the other clade was Catostominae. All deep nodes (i.e. catostomid tribes and higher taxonomic groupings) were supported by high Bayesian posterior probabilities (pp, 72–100%). Bootstrap support (bs) values from the RAxML analysis were slightly lower than Bayesian pp for the deeper nodes. The relationships of the catostomine tribes were as follows: (Erimyzonini, (Catostomini, (Thoburniini, Moxostomatini))).

In all analyses Ictiobinae was monophyletic, comprising a monophyletic *Ictiobus* sister to a monophyletic *Carpiodes*, with strong support. Relationships of species within *Ictiobus* and *Carpiodes* differed in trees based on the two data sets (Figs. 1 and 2). *Ictiobus* species are introgressed throughout wide portions of their range (Bart et al., in review) and ND4/ND5 sequence divergence among the four species was also quite low (0.66%). *Carpiodes* species were slightly more divergent than *Ictiobus* species (1.17%).
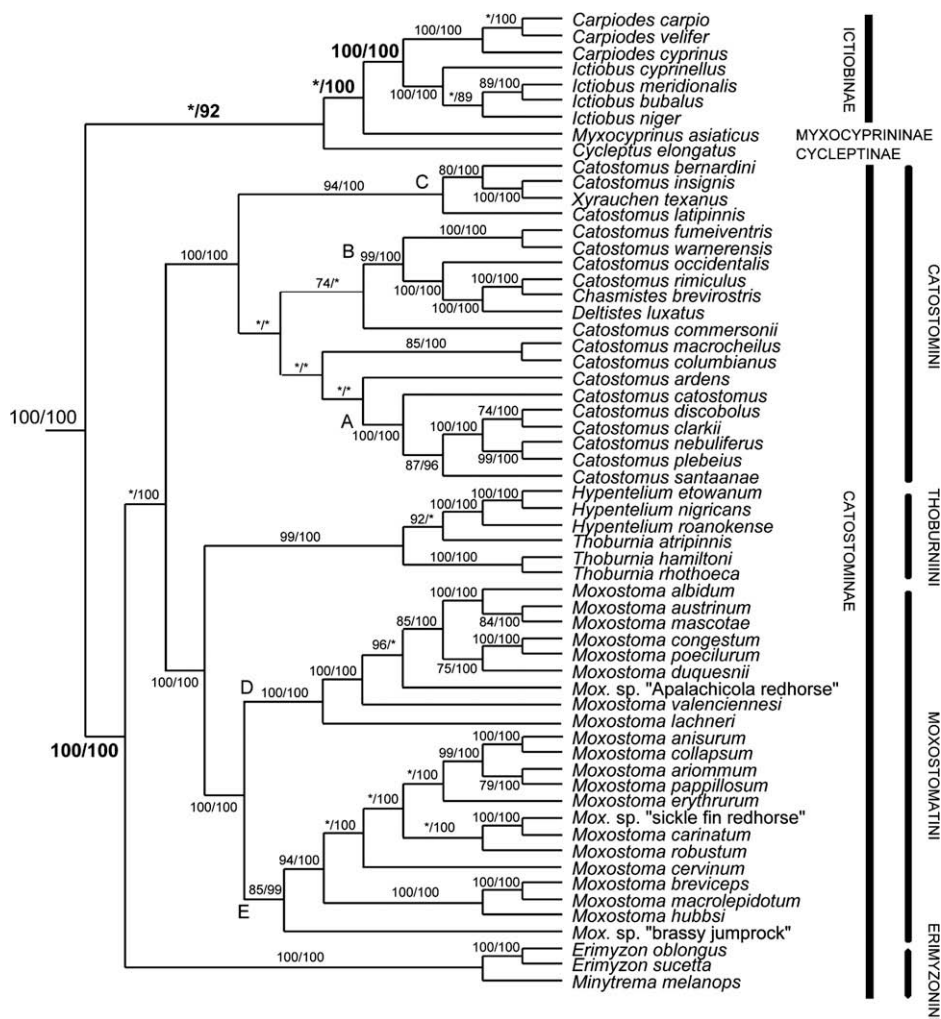

Fig. 1. The best ML tree of catostomid relationships inferred from the all substitution data set. Numbers at the nodes are bootstrap replicates of RAxML analysis (left) and Bayesian posterior probabilities (right). An asterisk indicates that support is less that 70%. Bold text indicates support for the subfamilies. See Section 3.2 for explanation of clades A–E.

Within the subfamily Catostominae, *Minytrema melanops* was basal and sister to *Erimyzon oblongus* plus *E. sucetta* with strong statistical support (100% bs and pp). However, ML bs support for the basal position of Erimyzonini was weak for both data sets. Only the Bayesian tree based on the all substitutions data set had significant support (100% pp) for the basal placement of Erimyzonini (Fig. 1).

In all analyses Catostomini was monophyletic and supported with 100% bs and pp, and interrelationships of the species were similar. *Catostomus* was not recovered as monophyletic because *Chasmistes*, *Deltistes*, and *Xyrauchen* were deeply nested with *Catostomus* species. Catostomine relationships were not completely congruent in analyses based on the two data sets; however, some species groups were recovered consistently with strong support. Clade A in Figs. 1 and 2 was recovered in all analyses and contained six species of *Catostomus* with the following relationships: (*catostomus*, (*santaanae*, ((*discobolus*, *clarkii*), (*nebuliferus*, *plebeius*)))). Clade B in Figs. 1 and 2 was recovered in all analyses and contained six species with high support ((*C. fumeiventris*, *C. warnerensis*), (*C. occidentalis*, (*Deltistes luxatus*, (*C. rimiculus*, *Chasmistes brevirostris*)))). Clade C in Figs. 1 and 2 was recovered in all analyses and contained four species with high support (*C. latipinnis*, (*C. bernardini*, (*C. insignis*, *Xyrauchen texanus*))). *Catostomus columbianus* was sister to *C. macrocheilus* in all analyses, but the sister group of this pair differed in the two trees. The relationships of *C. ardens* and *C. commersonii* to other catostomines are also ambiguous.

Thoburniini was recovered as a monophyletic group with strong support (98–100% bs and pp). All analyses resolved *Hypentelium* as a monophyletic group with 99–100% bs and pp support (Figs. 1 and 2). *Hypentelium roanokense* was basal and sister to the pair of *H. nigricans* plus *H. etowanum*. *Thoburnia* was not monophyletic because *T. atripinnis* was recovered as the sister to *Hypentelium* with strong support (91–100% bs and pp) in all analyses except the Bayesian analysis involving all substitutions. *Thoburnia hamiltoni* was sister to *T. rhothoeca* in both trees, and this pair was the basal group of Thoburniini.

Moxostomatini was recovered as a monophyletic group with strong support (99–100% bs and pp). Within the Moxostomatini, two major clades were resolved, but the relationships within these clades differed somewhat because of low support for some nodes. Nine species of *Moxostoma* were resolved (clade D in Figs. 1 and 2), comprising a basal *M. lachneri* which was sister to a group of eight species. The relationships of the species of clade D are identical in both best ML trees except for the placement of *M. valenciennesi* and the undescribed "Apalachicola redhorse." A strongly supported group of six derived species was resolved in both analyses. A trio of southwestern-Mexican species (*M. albidum*, (*M. austrinum*, *M. mascotae*)) was sister to a trio of more eastern species (*M. duquesnii*, (*M. congestum*, *M. poecilurum*)).

The other clade of *Moxostoma* species (clade E in Figs. 1 and 2) contains 13 species. Relationships among species in clade E are nearly consistent in both ML trees, with the exception of
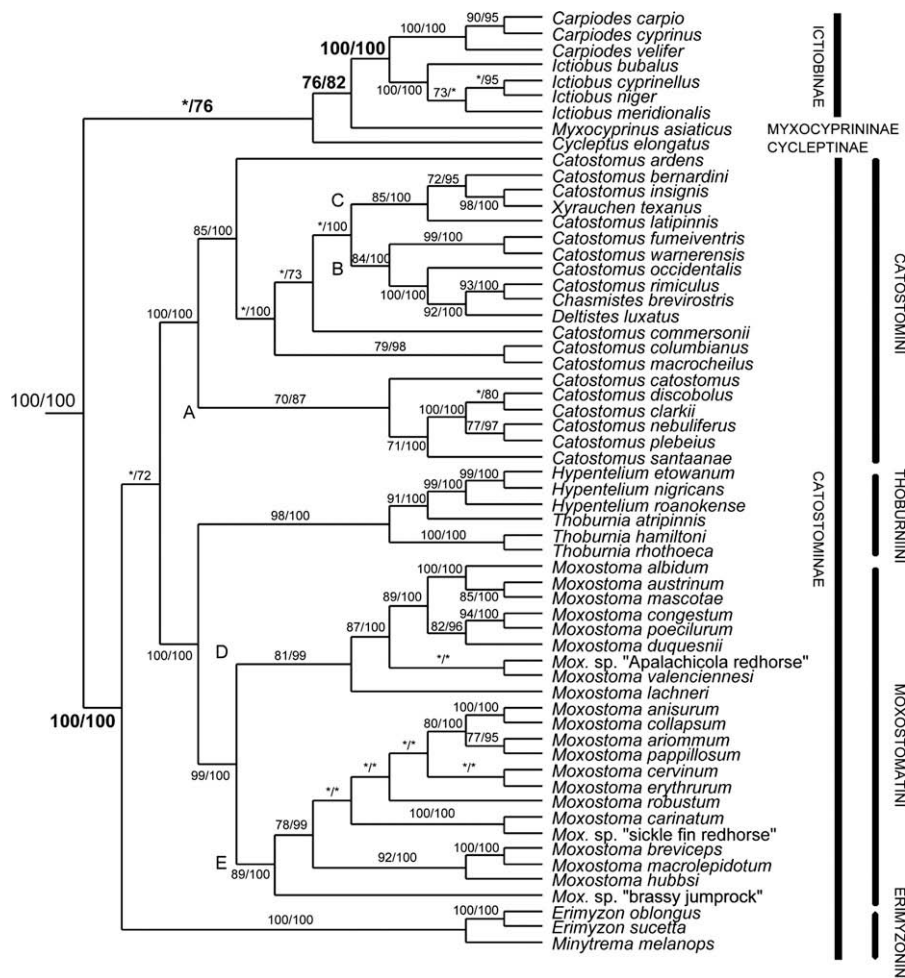

Fig. 2. The best ML tree of catostomid relationships inferred from the RY-coded data set. Numbers at the nodes are bootstrap replicates of RAxML analysis (left) and Bayesian posterior probabilities (right). An asterisk indicates that support is less that 70%. Bold text indicates support for the subfamilies. See Section 3.2 for explanation of clades A–E.

interrelationships of *M. cervinum*, *M. erythrurum*, and *M. robustum*. The undescribed "brassy jumprock" is the basal member of this clade. In the next group proceeding up the tree, *M. hubbsi* is consistently sister to *M. breviceps* plus *M. macrolepidotum*. Next, *Moxostoma carinatum* is sister to the undescribed "sickle fin redhorse." The most derived group of species in clade E is a strongly supported group comprising four species: ((*M. ariommum*, *M. pappillosum*), (*M. anisurum*, *M. collapsum*)). The placement of *M. ariommum* in a group with V-lip redhorses is a novel hypothesis that is consistently supported by ND4/ND5 data.

4. Discussion

Previous studies of catostomid phylogenetic relationships resolved Ictiobinae or Myxocyprininae as the most basal subfamily, and place Cycleptinae sister to Catostominae (Ferris and Whitt, 1978; Fuiman, 1985; Smith, 1992; Harris and Mayden, 2001). Phylogenetic analysis of ND4/ND5 data resolved catostomids as two major clades; one comprising Catostominae and the other comprising a basal Cycleptinae sister to Myxocyprininae plus Ictiobinae, a novel hypothesis of catostomid relationships. The only caveat is that most of the statistical support for this hypothesis comes from Bayesian support values; bootstrap support for grouping Cycleptinae, Ictiobinae and Myxocyprininae is comparatively low. Studies suggest that Bayesian pp tends to be high compared to other support measures and should not be interpreted as a high probability that a given tree is correct (Simmons et al., 2004).

Relationships of the ictiobine species differ from Smith's (1992) hypothesis. *Ictiobus niger* was never recovered as the basal species of genus *Ictiobus*, as proposed by Smith (1992). Trees derived from analysis of all ND4/ND5 substitutions corroborated the relationships of *Carpiodes* species as proposed by Smith (1992), whereas, the RY-coded data set has *C. velifer* as the basal taxon as recovered by Ferris and Whitt (1978).

Analyses of ND4/ND5 data consistently resolved Erimyzonini as the basal group of Catostominae, a novel hypothesis of relationships for the subfamily. Smith (1992) included *Erimyzon* and *Minytrema* as basal genera of Moxostomatini. Harris and Mayden (2001), Harris et al. (2002), and Sun et al. (2007) were uncertain of the position of Erimyzonini.

Sister to Erimyzonini was Catostomini plus Moxostomatini (including Thoburniini). ND4/ND5 data were consistent in supporting the monophyly of Catostomini. Some of the previous studies had placed Catostomini as a derived tribe sister to Moxostomatini (Ferris and Whitt, 1978; Smith, 1992); others placed Catostomini as the basal tribe of Catostominae (Harris and Mayden, 2001; Harris et al., 2002).

Smith (1992) resolved two reciprocally monophyletic clades of Catostomini: one comprising *Catostomus* species, and the other comprising *Xyrauchen texanus* sister to *Deltistes* plus *Chasmistes*. *Catostomus* species were poorly resolved in the analysis of all ND4/ND5 substitutions, but better resolved in the RY-coded data set. *Catostomus* was never recovered as a monophyletic group because of placement of *Chasmistes*, *Deltistes*, and *Xyrauchen* within *Catostomus*. There is evidence for a monophyletic subgenus *Pantosteus*; however, generic recognition of *Pantosteus* would render *Catostomus* paraphyletic (Smith, 1992).

Phylogenetic trees derived from mtDNA gene sequences do not support recognition of *Xyrauchen*, *Deltistes*, and *Chasmistes* as distinct from *Catostomus* (Harris and Mayden, 2001; Harris et al., 2002; this study). There is considerable evidence for hybridization among *Catostomus*, *Chasmistes*, and *Deltistes* (Harris et al., 2002; Miller and Smith, 1981; Smith, 1992). Hybridization may account for the clade containing *Deltistes luxatus* sister to *Chasmistes brevirostris* plus *Catostomus rimiculus* in the present study. A sister relationship of *Deltistes* and *C. rimiculus* was also found by Harris et al. (2002) using the same specimens, but different mitochondrial genes, as used for this study. However, their analysis did not include *Chasmistes brevirostris*. Therefore, the ND4/ND5 results either confirm the close relationship of *Deltistes* and *C. rimiculus* or provide another line of evidence for the hypothesis of hybridization between these taxa.

Moxostomatini is sister to Thoburniini with strong support in all ND4/ND5 analyses. *Thoburnia rhothoeca* is sister to *T. hamiltoni* in all analyses, confirming hypotheses of previous workers (Buth, 1979; Jenkins, 1970; Smith, 1992). However, *T. atripinnis* is sister to *Hypentelium* in both best ML trees, the same result was obtained in Harris et al.'s (2002) cyt *b* analysis and Smith's (1992) comprehensive analysis, suggesting that *T. atripinnis* is misclassified as a species of *Thoburnia*. *Hypentelium etowanum* is sister to *H. nigricans,* in agreement with (Ferris and Whitt, 1978; Harris et al., 2002; Jenkins, 1970), but differing from Smith (1992), who recovered *H. etowanum* basal and sister *to H. roanokense* plus *H. nigricans*.

Moxostomatini is found to be the most derived tribe of the Catostominae in all analyses. Similar to Harris et al.'s (2002) cyt *b* analysis, the ND4/ND5 data set provides no support for recognizing a monophyletic genus *Scartomyzon,* separate from *Moxostoma*. However, relationships within *Moxostoma* in the present study differ from Harris et al. (2002). Two major clades were recovered within *Moxostoma* in the present analysis, with similar species groupings, but better resolution (i.e. fewer polytomies) than in the cyt *b* tree of Harris et al. (2002). An unexpected result in the ND4/ND5 trees was inclusion of *M. ariommum* in a clade with the V-lip redhorse species group (*M. pappillosum*, *M. anisurum*, and *M. collapsum*). Earlier workers noted that *M. ariommum* was either intermediate between *Scartomyzon* and *Thoburnia* (Bailey, 1959; Robins and Raney, 1956) or related to *T. atripinnis* (Jenkins, 1970). *Moxostoma ariommum* was sister to the *M. albidum* group with varying support (61–82% bs) in Harris et al. (2002). Smith's (1992) tree placed *M. ariommum* in a polytomy with *Thoburnia* and *Hypentelium*.

Saturation was only detectable in third position transitions (Supplementary Fig. S1). The tree based on all ND4/ND5 substitutions is better resolved at its tips (especially within Moxostomatini) than the tree based on RY-coded data. RY-coding may have reduced saturational noise at deeper nodes (e.g. cypriniform families and catostomid subfamilies) in the ML tree, but it clearly also reduced phylogenetic signal at more superficial levels within Catostomidae. The signal to noise ratio was likely higher in trees of Harris and Mayden (2001) and Harris et al. (2002), which involved analysis of all substitutions of more variable mitochondrial genes (Miya et al., 2006).

The results of this study have contributed novel hypotheses of catostomid relationships. However, further evidence is needed to clarify the ambiguous relationships of catostomid subfamilies. Further investigation, preferably involving nuclear genes, is also required to elucidate relationships within Catostominae, particularly the taxonomic status and sister taxa of *Chasmistes*, *Deltistes*, and *Xyrauchen*. The systematic position and taxonomic status of *Thoburnia atripinnis* is also problematic. Lastly, relationships of *Moxostoma* species are not yet fully resolved, and there is much alpha taxonomic work to be completed. Complete understanding of catostomid relationships must await assembly of additional DNA sequence data (e.g. whole mitogenomes and multiple nuclear loci) and corroborating morphological evidence.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.06.006.

References

Bailey, R.M., 1959. A new catostomid fish, Moxostoma (Thoburnia) atripinne, from the Green River drainage, Kentucky and Tennessee. Occasional Papers of the Museum of Zoology, The University of Michigan 599, pp. 1–19.

Bart Jr., H.L., Clements, M.D., Blanton, R.E., Piller, K.R., Hurley, D.L., in review. Discordant morphological and molecular evolution in buffalofishes (Actinopterygii: Catostomidae). Evolution.

Buth, D.G., 1979. Genetic relationships among torrent suckers, genus Thoburnia. Biochemical Systematics and Ecology 7, 311–316.

Ferris, S.M., Whitt, G.S., 1978. Phylogeny of tetraploid catostomid fishes based on the loss of duplicate gene expression. Systematic Zoology 27, 189–206.

Fuiman, L.A., 1985. Contributions of developmental characters to a phylogeny of catostomid fishes, with comments on heterochrony. Copeia 1985, 833–846.

Harris, P.M., Mayden, R.L., 2001. Phylogenetic relationships of major clades of Catostomidae (Teleostei: Cypriniformes) as inferred from mitochondrial SSU and LSU rDNA sequences. Molecular Phylogenetics and Evolution 20, 225–237.

Harris, P.M., Mayden, R.L., Perez, H.S.E., Garcia de Leon, F., 2002. Phylogenetic relationships of Moxostoma and Scartomyzon (Catostomidae) based on mitochondrial cytochrome b sequence data. Journal of Fish Biology 61, 1433–1452.

Hubbs, C.L., 1930. Materials for a revision of the catostomid fishes of eastern North America. Miscellaneous Publications. Museum of Zoology, University of Michigan 20, pp. 5–47.

Jenkins, R.E., 1970. Systematic Studies of the Catostomid Fish Tribe Moxostomatini. Cornell University, Ithaca, NY. p. 799.

Kumazawa, Y., Nishida, M., 1993. Sequence evolutions of mitochondrial tRNA genes and deep-branch animal phylogenetics. Journal of Molecular Evolution 37, 380–398.

Mabuchi, K., Miya, M., Senou, H., Suzuki, T., Nishida, M., 2006. Complete mitochondrial DNA sequence of the Lake Biwa wild strain of common carp (Cyprinus carpio L.): further evidence for an ancient origin. Aquaculture 257, 68–77.

Mayden, R.L., Chen, W.-J., Bart Jr., H.L., Doosey, M.H., Simons, A.M., Tang, K.L., Wood, R.M., Agnew, M.K., Yang, L., Hirt, M.V., Clements, M.D., Saitoh, K., Sado, T., Miya, M., Nishida, M., 2009. Reconstructing the phylogenetic relationships of the Earth's most diverse clade of freshwater fishes – order Cypriniformes (Actinopterygii: Ostariophysi): a case study using multiple nuclear loci and the mitochondrial genome. Molecular Phylogenetics and Evolution 51, 500–514.

Miller, R.R., Smith, G.R., 1981. Distribution and evolution of Chasmistes (Pisces: Catostomidae) in western North America. Occasional Papers of the Museum of Zoology, The University of Michigan 696, pp. 1–46.

Miya, M., Saitoh, K., Wood, R., Nishida, M., Mayden, R.L., 2006. New primers for amplifying and sequencing the mitochondrial ND4/ND5 gene region of the Cypriniformes (Actinopterygii: Ostariophysi). Ichthyological Research 53, 75–81.

Murakami, M., Yamashita, Y., Fujitani, H., 1998. The complete sequence of mitochondrial genome from a gynogenetic triploid "ginbuna" (Carassius auratus langsdorfi). Zoological Science 15, 335–337.

Nelson, J.S., 2006. Fishes of the World. John Wiley and Sons, New York.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Robins, C.R., Raney, E.C., 1956. Studies of the catostomid fishes of the genus Moxostoma, with descriptions of two new species. Cornell Agricultural Experiment Station Memoir 343, 3–56.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Saitoh, K., Miya, M., Inoue, J.G., Ishiguro, N.B., Nishida, M., 2003. Mitochondrial genomics of ostariophysan fishes: perspectives on phylogeny and biogeography. Journal of Molecular Evolution 56, 464–472.

Saitoh, K., Sado, T., Mayden, R.L., Hanzawa, N., Nakamura, K., Nishida, M., Miya, M., 2006. Mitogenomic evolution and interrelationships of the Cypriniformes (Actinopterygii: Ostariophysi): the first evidence toward resolution of higher-level relationships of the world's largest freshwater fish clade based on 59 whole mitogenome sequences. Journal of Molecular Evolution 63, 826–841.

Simmons, M.P., Pickett, K.M., Miya, M., 2004. How meaningful are Bayesian support values? Molecular Biology and Evolution 21, 188–199.

Smith, G.R., 1966. Distribution and evolution of the North American catostomid fishes of the subgenus Pantosteus, genus Catostomus. Miscellaneous Publications. Museum of Zoology, University of Michigan 129, pp. 1–132.

Smith, G.R., 1992. Phylogeny and biogeography of the Catostomidae, freshwater fishes of North America and Asia. In: Mayden, R.L. (Ed.), Systematics, Historical Ecology, and North American Freshwater Fishes. Stanford University Press, Stanford, CA, pp. 778–826.

Smith, G.R., Koehn, R.K., 1971. Phenetic and cladistic studies of biochemical and morphological characters of Catostomus. Systematic Zoology 20, 282–297.

Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 57, 758–771.

Sun, Y.H., Xie, C.X., Wang, W.M., Liu, S.Y., Treer, T., Chang, M.M., 2007. The genetic variation and biogeography of catostomid fishes based on mitochondrial and nucleic DNA sequences. Journal of Fish Biology 70 (Suppl. C), 291–309.

Swofford, D.L., 2002. PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods), fourth ed. Sinauer Associates, Sunderland, MA.

Uyeno, T., Smith, G.R., 1972. Tetraploid origin of the karyotype of catostomid fishes. Science 175, 644–646.