Phylogenetic relationships of suckers of the subfamily Ictiobinae (Teleostei: Catostomidae) as inferred from Cytochrome b sequence data

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1   Suckers of the subfamily Ictiobinae are large, deep-bodied fishes adapted to large rivers

2   and lakes. Seven extant species are currently recognized in two genera: *Carpiodes* and *Ictiobus*.

3   A comprehensive phylogenetic analysis involving osteological, allozymic, developmental and

4   external morphological characters found ictiobines to be the most basal catostomids and resolved

5   relationships within the subfamily (Smith 1992). A more recent phylogeny based on

6   mitochondrial DNA sequence data (Harris and Mayden 2001) suggests that the Asian sucker,

7   *Myxocyprinus asiaticus*, is the most basal catostomid, with ictiobines sister to all other

8   Catostomids. We sequenced the entire mitochondrial cytochrome b gene (*cyt b*) for 20

9   specimens representing all of the currently recognized ictiobine species, and outgroups

10   representing other catostomid subfamilies and groups from which catostomids are postulated to

11   have evolved. Phylogenetic trees were generated using maximum parsimony and Bayesian

12   maximum likelihood analyses. Both analyses resolve the two genera of ictiobines as

13   monophyletic and show *I. labiosus* to be the most divergent species of *Ictiobus*. However, for

14   some species of *Carpiodes* and *Ictiobus,* sequences failed to group consistently with the

15   morphological identity of specimens they were obtained from. Patterns of *cyt b* sequence

16   variation in these instances suggest that species of *Carpiodes* and *Ictiobus* are hybridizing where

17   sympatric in Mississippi River Basin. Trees based on *cyt b* sequence data are most similar to

18   Smith's (1992) hypothesis of basal relationships of catostomids.

19

20   SUCKERS of the subfamily Ictiobinae are large, deep-bodied, large-scaled fishes adapted

21   to large rivers and lakes. The subfamily has a Holarctic distribution, but is represented only by

22   fossils of Paleocene to Oligocene age west of the Continental Divide in North America and

1   eastern Asia (†*Amyzon* spp. and †*Vasnetzovia* sp.). Living forms and more recent fossils

2   (Miocene to Recent) of the genera *Carpiodes* and *Ictiobus* are naturally found only east of the

3   Continental Divide in North America, Mexico and northern Guatemala. Seven or eight extant

4   species of ictiobines are currently recognized: *Carpiodes carpio* (Rafinesque) *C. cyprinus*

5   (LeSueur), *C. velifer* (Rafinesque); *Ictiobus bubalus* (Rafinesque), *I. cyprinellus* (Valenciennes),

6   *I. labiosus* (Meek), *I. meridionalis* (Gunther) and *I. niger* (Rafinesque).   Recent workers regard

7   *I. meridonalis* as synonymous with *I. bubalus* (Mayden et al., 199; Smith, 1992; Miller, *in*

8   *review*).

9   A comprehensive phylogenetic analysis involving osteological, allozymic, developmental

10   and external morphological characters found ictiobines to be the most basal catostomids and

11   resolved relationships within the subfamily (Smith, 1992; Fig. 1). Since the oldest fossil

12   catostomids are ictiobines, this result is consistent with the fossil record.   Moreover, it suggests

13   that the body plan of ictiobines - which resembles that of the Asian cyprinids that suckers are

14   believed to have evolved from (Uyeno and Smith 1972) - is pliesiomorphic (Smith, 1992).

15   Smith's (1992) phylogenetic hypothesis suggests that ictiobines are key to understanding early

16   catostomid evolution. The "preferred hypothesis" from a recent study of relationships of major

17   catostomid clades based on mitochondrial ribosomal DNA sequence data (Harris and Mayden,

18   2001) suggests that the Asian sucker, *Myxocyprinus asiaticus*, is the most basal catostomid, with

19   ictiobines sister to all other Catostomids.

20   Development of DNA sequencing technology has provided systematists a powerful new

21   tool for elucidating evolutionary relationships among species (Hillis et al., 1996).   The

22   mitochondrial genome is the best studied of all types of DNA (Kocher and Carleton, 1997).   Its

23   rapid rate of evolution, clonal inheritance, and lack of recombination have made it a valuable

1 resource for studies of intraspecific phylogeography and gene flow (Avise, 1994; Kocher and

2 Stepien, 1997), delineation of species boundaries (Frost et al., 1998; Eitner et al., 1999; Tanka-

3 Ueno et al., 1999), and phylogeny reconstruction (Briolay et al., 1998; Harris and Mayden,

4 2001). The cytochrome-b gene (hereafter *cyt b*) is the best-studied mitochondrial gene,

5 especially in fishes (Lydeard and Roe, 1997). The gene has both conserved and variable regions,

6 and is thus useful for investigating relationships of both closely and distantly related species

7 (Lydeard and Roe, 1997).

8   In this study we describe sequence variation in *cyt b* for living ictiobines and outgroups

9 representing the other catostomid subfamilies and groups from which catostomids are postulated

10 to have evolved (namely cyprinids and cobitids, Smith, 1992). We use *cyt b* sequence data to

11 infer phylogenetic relationships among ictiobines and other catostomid species. We use evidence

12 from the fossil record to estimate minimum divergence dates and rates of base substitution for

13 extant groups. Lastly, we compare our results to the phylogenetic hypotheses of Smith (1992)

14 and Harris and Mayden (2001).

15

16   MATERIALS AND METHODS

17   Complete *cyt b* sequence data were been obtained for 20 individuals representing all of

18 the currently recognized extant species of ictiobines, including *I. meridionalis* from Río

19 Usumacinta, Mexico (Table 1). A number of species, and populations within some species, are

20 represented by multiple individuals.

21   Total genomic DNA was extracted from frozen or ethanol preserved tissues using the

22 DNeasy Tissue Kit (Qiagen, Inc.). The 1,140 bp *cyt b* gene was isolated by polymerase chain

23 reaction (PCR). We use the oligonucleotides GLU (5'-TAA CCG AGA CCA ATG ACT TG-3')

1   and THR (5'-ATC TTC GGA TTA CAA GAC CG-3', Brady Porter unpublished) to amplify the

2   gene. Ten additional internal sequencing primers were designed with OLIGO primer analysis

3   software (Molecular Biology Insights) to sequence the gene for ictiobines and outgroups (primer

4   sequences available from the senior author on request). Two of the sequencing primers used for

5   *Carpiodes* differed from those used for *Ictiobus* and outgroups.

6   Reactions were cycled according to the following temperature profile: 94°C for 1 min.,

7   57°C for 1 min., and 72°C for 1:15 min., for 32 cycles. PCR products were isolated with the

8   QIAquick PCR Purification Kit (Qiagen) and used in cycle sequencing reactions (Applied

9   Biosystems) according to the manufacturer's recommendations. Excess dye terminators, primers,

10   and nucleotides were removed by gel filtration (Edge Biosystems) prior to sequencing. Complete

11   bidirectional sequences of *cyt b* were obtained with an ABI 373A Automated Sequencer.

12   Reactions were electrophoresed on 6% polyacrylamide gels in 7 M urea (Sooner Scientific).

13   Raw sequence chromatograms of approximately 400 bp length were assembled into

14   contigs and edited to resolve ambiguities using Sequencer 4.1 (Gene Codes). Sequences were

15   aligned to *Myxocyprinus asiaticus*, an Asian Cycleptine (GenBank AF036176). Amino acid

16   sequences were determined and analyzed with MacVector 4.0 (Oxford Molecular).

17   Sequences were compared for all pairs of taxa to determine numbers of variable sites and

18   to distinguish transition substitutions from transversion at each codon position. Transitions and

19   transversions were plotted against uncorrected sequence divergence to check for evidence of

20   saturation (Fig. 2). Sequence divergences were recomputed as "corrected" Tamura and Nei (T-

21   N) distances to adjust for saturation effects (Tamura and Nei, 1993). T-N distances were used to

22   construct a neighbor-joining (NJ) tree with branch length denoting divergence among taxa (Fig.

23   3).

1   Phylogenetic trees were generated with PAUP (vers 4.0b10, D.L. Swofford, Sinauer

2   Associates, Sunderland, MA, 1999, unpublished) using maximum parsimony (MP) and

3   maximum likelihood (ML) optimality criteria. MP analysis employed heuristic searches (TBR

4   branch swapping; MULTREES option in effect) with 10 random stepwise additions of taxa.

5   Robustness of inferred nodes was assessed with bootstrap analysis (1000 pseudoreplicates). ML

6   analysis used the GTR+G+I model of nucleotide substitution. The best fitting model was

7   determined by a likelihood ratio test using the program MODELTEST 3.04 (Posada and

8   Crandall, 1998). Heuristic searches were then conducted in PAUP (same parameters as MP) to

9   determine the best tree topology. Posterior probabilities (i.e. confidence) of nodes in ML trees

10   were estimated using MRBAYES (Huelsenbeck and Rondquiust, in press) The Bayesian analysis

11   started with a random tree and used a Markov chain Monte Carlo process (actually, four chains

12   run simultaneously) to resample tree topology and ML parameters every 100 generations for a

13   total of 1,500,000 generations. Log likelihood scores stabilized after approximately 30,000

14   generations (3,000 trees). Thus, the "burnin" value in MRBAYES was set at 3,000 and the first

15   3,000 trees were ignored in determining the consensus tree topology and posterior probabilities

16   of its nodes.

17   Alternate topologies in MP and ML trees were tested using Templeton's (1983) and

18   Shimodoira and Hasegawa's (1999) topological tests as implemented in PAUPb10.

19

20   RESULTS

21   *Sequence variation*

22   Complete *cyt b* sequence data (1,140 bp) were generated for all ingroup taxa and five of

23   the outgroup taxa in Table 1. Sequences for the remaining outgroups were obtained from

1   Genbank. At the level of all taxa, the sequences contained 478 variable sites. Eighty-five percent

2   (85%) of the substitutions were at the third codon position, 12% were at the first codon position,

3   3% at the second codon position. Transitions out numbered transversions 2.5:1. At the level of

4   all ictiobines, 172 sites were variable. Ninety-four percent (94%) of the substitutions were at the

5   third codon position; the remaining 6% were at the first codon position. The transition to

6   transversion ratio (Tr/Tv) was 7:1. Within genus *Ictiobus* 74 sites were variable; 89% of the

7   substitutions were at the third codon position, and transitions outnumbered transversions 20:1.

8   There were 28 variable sites within genus *Carpiodes*. All but one of the substitutions was at the

9   third codon position; the transition/transversion ratio was 47:1. Sequences showed evidence of

10 saturation only for transitions at the third codon position Fig. 2). All of the sequences showed

11 the distinct anti-G bias at the second and third codon positions typical of mitochondrial genes.

12   None of the base substitutions in genus *Carpiodes* altered the amino acid sequence of the

13 *cyt b* protein. Four amino acid differences were noted for *Ictiobus* sequences. The *I. labiosus*

14 sequence coded for Alanine at amino acid position 327, whereas all other ictiobines in the

15 analysis had Threonene at this position (a non-conservative change). The *I. labiosus* sequence

16 also coded for Isoleucine at position 364, whereas all other ictiobines had Valine (a conservative

17 substitution). All *I. bubalus* populations and *I. meridionalis* shared the hydrophobic amino acid

18 Valine at position 333, whereas all other ictiobines had Isoleucine (also hydrophobic). Finally,

19 the Wisconsin River *I. cyprinella* sequence supported a non-conservative change from Isoleucine

20 to Alanine at position 360.

21   Sequence divergences among taxa are described below and depicted graphically (T-N

22 distances for catostomids only) in Fig 3. Divergence among *Carpiodes* species ranges from 0-

23 1.3% (mean = 0.85%). *Cyt b* sequences for the two *C. velifer* specimens are identical. Sequences

1 for *C. cyprinus* specimens from the Ohio and the Upper Mississippi rivers, the "*C. forbesi*"

2 specimen, and two of the four *C. carpio* specimens (Ohio and Upper Mississippi R.) differ at

3 only two positions (0.18%), suggesting that all are variants of the typical Mississippi River Basin

4 *C. cyprinus* haplotype. The fact that two specimens with unmistakable *C. carpio* morphotypes

5 show this haplotype, suggests that the specimens are maternal *C. cyprinus* x paternal *C. carpio*

6 hybrids. *Cyt b* sequences for two other *C. carpio* specimens (Ohio and Lower Mississippi R.)

7 are 0.96 to 1.16% divergent from the typical Mississippi River Basin *C. cyprinus* haplotype, 0.43

8 to 0.61% divergent from the *C. velifer* haplotype, and 0.35% divergent from each other. We

9 regard sequences of these two latter *C. carpio* specimens as more typical of the true *C. carpio cyt*

10 *b* haplotype. *Cyt b* sequences for the two *C. velifer* specimens are 1.24% divergent from the

11 Mississippi River Basin *C. cyprinus* haplotype, and 1.06% divergent from sequences for the

12 putative *C. carpio* x *cyprinus* hybrids.   Finally, the *cyt b* sequence from the Atlantic Slope

13 (James River) *C. cyprinus* specimen is 1.15% divergent from the typical Mississippi River Basin

14 *C. cyprinus* haplotype, and 1.24-1.33% divergent from *cyt b* sequences for *C. velifer* and typical

15 *C. carpio*.

16   *Cyt b* sequence divergence among *Ictiobus* species averages 1.51%. However, much of

17 this is due to the high degree of divergence of *I. labiosus* from other *Ictiobus* species (average of

18 6%). Divergences among *I. bubalus*, *I. cyprinellus* and *I. niger* average 0.46%. Divergence

19 within each these latter three species - even among widely separated populations of *I. bubalus*

20 (inclusive of *I. meridionalis*) - is generally much lower. *Cyt b* sequences for *Ictiobus bubalus*

21 specimens from the Río San Fernando in northeastern Mexico and the Amite River in Louisiana

22 (Lake Pontchartrain Basin) are identical. Sequences for the *I. meridionalis* specimen from Río

23 Usumacinta (extreme southeastern Mexico) and *I. bubalus* from the Upper Mississippi River

1   differ by only one base. Mean sequence divergence within *I. bubalus* (inclusive of *I.*

2   *meridionalis*) is 0.22%. Two base substitutions separate the sequences of Ohio and Upper

3   Mississippi R. specimens of *I. niger* (a divergence of 0.17%), and only one base substitution

4   separates the sequences of Ohio and Upper Mississippi R. specimens of *I. cyprinellus*.

5   The *cyt b* sequence for the Amite River specimen of *I. cyprinellus* is unusual in that it is

6   0.57% divergent on average from other *I. cyprinellus* sequences, but only 0.09% and 0.25%

7   divergent, respectively, from upper Mississippi and Ohio R. *I. niger cyt b* sequences, suggesting

8   that the Amite River *I. cyprinellus* specimen is expressing an *I. niger cyt b* DNA haplotype.

9   *Cyt b* sequence divergences among *Carpiodes* and *Ictiobus* species average 11.6%

10   overall, but are slightly higher for comparisons involving *Carpiodes* species and *I. labiosus*

11   (average of 13%), than those involving *Carpiodes* and other *Ictiobus* (average of 11.4%).

12   Average *cyt b* sequence divergence between *Carpiodes* species and the two Cycleptines

13   (*Myxocyprinus asiaticus* and *Cycleptus elongatus*) are similar (16.8 and 16.5%, respectively).

14   However, divergences between *Myxocyprinus asiaticus* and *Ictiobus* species are lower (average

15   of 15.6%) than divergences between *Cycleptus elongatus* and *Ictiobus* species (18.8% for *I.*

16   *labiosus*; average of 18% for other *Ictiobus*). As a group, Ictiobines are as divergent from

17   Moxostomini as from minnows (20.5 vs. 20.1%). Ictiobines are most divergent from loaches

18   among outgroups (24.5%). Catostomids are roughly 20-23% divergent from minnows and 24.4-

19   25.3% divergent from loaches.

20

21   *Phylogenetic analyses*

22   Of 478 variable sites in the *cyt b* gene at the level of all taxa, 416 are parsimony

23   informative. At the level of ictiobines, 131 of the172 variable sites are parsimony informative.

1   However, phylogenetic signal falls off considerably within ictiobine genera. Within genus

2   *Carpiodes*, 14 of the 28 variable sites are parsimony informative. In genus *Ictiobus*, only 8 of 74

3   variable sites are parsimony informative.

4   Unweighted maximum parsimony analysis resulted in 16 equally parsimonious trees of

5   1249 steps (CI = 0.52, RI = 0.73). Choice of outgroups (minnows, loaches or both) had no effect

6   ingroup relationships. The consensus tree based on 1000 bootstrap replicates is shown in Fig 4.

7   The analysis resolves subfamily Ictiobinae and genera *Carpiodes* and *Ictiobus* as monophyletic

8   with high bootstrap support. Tribe Moxostomini and Family Catostomidae are also resolved as

9   monophyletic with strong bootstrap support (100% and 95% respectively). There is less support

10   for subfamily Cycleptinae (*Cycleptus elongatus* plus *Myxocyprinus asiaticus,* 48%), and a sister

11   relationship between subfamilies Cycleptinae and Ictiobinae (75%).

12   We restricted the analysis to 1st and 2nd position substitutions plus 3rd position

13   transversions to avoid the potential for homoplasy due to saturation of 3rd position transitions.

14   In the resulting tree (not shown), ictiobines are sister to a group comprising *Myxocyprinus*

15   *asiaticus* plus *Cycleptus elongatus* and Moxostomini. Using minnows as outgroups to

16   catostomids in this latter analysis groups resulted in shorter trees than when cobitids are used

17   (328 steps versus 344 steps), but has no effect on ingroup relationships.

18   The likelihood ratio test in Modeltest identified GTR+I+G as the model of base

19   substitution that best fit the data. The model derived Ti/Tv ratio was 9.26:1, proportion of

20   invariate sites (I) was 0.554, and gamma distribution shape parameter (G) was 1.08. The tree

21   resulting from Bayesian analysis of this dataset (Fig. 5) is identical to the result obtained through

22   MP analysis of the limited dataset (monophyletic Ictiobinae sister to a paraphyletic Cycleptinae

23   plus Moxostomini).

1   Interrelationships within Subfamily Ictiobinae are essentially the same in both of the

2   above analyses (compare Figs. 4 and 5). Within genus *Carpiodes*, *C. cyprinus* from the James

3   River is either sister to a group comprising all Mississippi River system *Carpiodes* (MP analysis,

4   Fig. 4) or forms an unresolved trichotomy with two groups of Mississippi River system

5   *Carpiodes*: one group comprising lower Mississippi and Ohio river specimens of *C. carpio*, plus

6   the two *C. velifer* specimens; the other group comprising upper Mississippi and Ohio River *C.*

7   *cyprinus* (including *C. forbesi*) plus putative hybrid upper Mississippi and Ohio river *C. carpio x*

8   *cyprinus* specimens (ML analysis, Fig. 5). Mississippi River *Carpiodes* form the same two

9   groups in the MP analysis. When *Carpiodes* species are forced to be monophyletic, the resulting

10   trees are significantly longer than the best MP tree topology (1259 vs. 1249 steps, Table 2).

11   Most of the added steps involve reversals in putative *C. carpio x cyprinus* hybrids.

12   Within genus *Ictiobus* in both MP and ML analyses, *I. labiosus* is sister to a group

13   comprising all other *Ictiobus* specimens, which is resolved as a polytomy consisting of the Amite

14   River specimen of *I. cyprinellus*, Ohio and Upper Mississippi river populations of *I. niger*, and a

15   group comprising all *I. bubalus* specimens (including *I. meridionalis*), plus Ohio and upper

16   Mississippi river specimens of *I. cyprinellus*. *Ictiobus cyprinellus* and *I. niger* specimens are not

17   resolved as monophyletic in either of the above analyses. However, constraining *Ictiobus*

18   species to be monophyletic does not significantly alter tree length (Table 2).

19

20   DISCUSSION

21   Cavender (1986) assigned fossil catostomids from the middle Miocene of South Dakota

22   to genus *Ictiobus*, suggesting that the genus diverged at least 15 mya. Using this value as the

23   minimum age of the divergence of *Ictiobus* and *Carpiodes*, with the average *cyt b* sequence

1   divergence between species in these genera (11.6%) gives a rate of *cyt b* sequence divergence of

2   0.78% per million years. Applying this rate of divergence to the divergence of *I. labiosus* from

3   the common ancestor of other *Ictiobus* would put the date of this divergence at 4.5 mya (lower

4   Pliocene). C. L. Smith (1962) identified fossils from the lower Pliocene of Oklahoma as *I*. cf.

5   *bubalus*, suggesting that the immediate ancestor of this species diverged roughly the same time

6   as *I. labiosus*.   Fossil remains agreeing with *I. cyprinellus* were identified in pre-glacial, early

7   Pleistocene deposits of Nebraska (1-1.5 mya; Smith and Lundberg ,1972). All other fossil

8   evidence of extant ictiobine species (*Carpiodes carpio*, *C. cyprinus*, *I. niger*) is from late

9   Pleistocene to Recent deposits (<1 mya, Smith 1981). *Cyt b* sequence divergences for *Carpiodes*

10 species are consistent with the ages of fossils of these species and suggest that they diverged

11 within the last 1 mya.   However, *cyt b* sequence divergences for *Ictiobus* species (exclusive of *I.*

12 *labiosus*) are much lower than expected based on fossil evidence.

13   All of the currently recognized ictiobine species (except perhaps *I. meridionalis*) are

14 diagnosable by a number of morphological characters. Indeed, most of the nominal species are

15 regarded as species complexes (Mayden et al., 1992; Mettee et al., 1996; Suttkus and Bart, 2002;

16 Bart and Suttkus, unpublished data). The fact that we were able to identify two cases in which

17 individuals with the morphotype of one species expressed the *cyt b* sequence pattern of a

18 sympatric congener, suggests that interspecific hybridization is influencing *cyt b* sequence

19 variation within ictiobine genera.

20   The hypothesis that hybridization is influencing patterns of *cyt b* sequence variation in

21 ictiobines is entirely plausible. Most of the species occur sympatrically across broad areas of

22 their ranges. Hybrids among *I. bubalus*, *I. cyprinellus*, and *I. niger* have been observed in nature

23 (Robison and Buchanan, 1989; Etnier and Starnes, 1993) and produced in experimental ponds

1   (Stevenson, 1964). Moreover, all three of these species are known to hybridize introgressively

2   in reservoirs (Johnson and Minckley, 1969). Hybridization is not as well documented among

3   *Carpiodes* species, but would explain our observation of *C. cyprinus cyt b* haplotypes in

4   specimens of *C. carpio* and an *I. niger* haplotype in a specimen of *I. cyprinellus*. This would also

5   explain the closer relationship of Mississippi River Basin populations of *C. cyprinus* to syntopic

6   populations of *C. carpio* and *C. velifer*, than to allopatric (Atlantic slope) *C. cyprinus*.

7   Degrees of mtDNA introgression cannot be assessed with the level of population

8   sampling involved in this study.   However, the fact that all of the species are maintaining their

9   morphological identity suggests that introgression is limited. Perhaps, interspecific

10   hybridization among ictiobines is a recent phenomenon brought about by human modification of

11   the large rivers these fishes inhabit. Virtually all of the rivers inhabited by these species have

12   been extensively modified for navigation, reservoir construction and other human uses. The

13   modifications disturb habitat, alter flow regime, restrict movements, and may interfere with

14   natural reproductive isolating mechanisms.

15   Concerns about interspecific hybridization prevent us from interpreting too much about

16   relationships within *Carpiodes* and *Ictiobus*, especially where species occur syntopically.

17   However, the following qualified conclusions are reasonably well supported by our data and

18   analyses. If we are correct in interpreting *cyt b* sequences of Licking and Wisconsin River

19   specimens of *C. carpio* as more typical of *C. cyprinus*, and sequences of Green and Sunflower

20   river specimens of *C. carpio* as more typical of the true *C. carpio cyt b* haplotype, and then our

21   trees suggest that *C. carpio* is more closely related to *C. velifer* than to *C. cyprinus*. This

22   conclusion is consistent with morphology and the phylogenetic hypothesis of Smith (1992).

23   We included samples from two syntopic, morphological variants of *C. cyprinus* from the

1   upper Mississippi River: a short and deep bodied, long-quilled form, which we regarded as the

2   typical upper Mississippi River quillback; and an elongate, short-quilled specimen, which agrees

3   with a "plains" form referred to in early literature as *C. forbesi* (Hubbs, 1930). The *cyt b* results

4   provide little support for the distinctiveness of the *C. forbesi*-like specimen from any other

5   Mississippi River specimen of *C. cyprinus*.

6   Our trees resolve all *I. bubalus* specimens, including *I. meridionalis*, as monophyletic.

7   *Cyt b* sequence data provide no support for the validity of *I. meridionalis*, a view shared by

8   Smith (1992) and Miller (in review). The very low *cyt b* sequence divergences, even among

9   widely disjunct populations, suggest that the populations are recently diverged. Buffalo suckers

10   are tolerant of and even spawn in brackish water (Perry, 1976). *Ictiobus bubalus* is frequently

11   taken in coastal lagoons in the U.S. and Mexico, and may be actively dispersing through coastal

12   waterways and low salinity estuaries. The specimen we sequenced from the Río San Fernando

13   was taken in water with a salinity of 4 parts per thousand.

14   Lastly, our results suggest that *I. labiosus*, is the most basal and divergent species of

15   *Ictiobus*. Smith's (1992) total evidence tree (Fig. 1) has *I. labiosus* sister to *I. cyprinellus* as one

16   of the more derived species of *Ictiobus*. The basal position of *I. labiosus* in our *cyt b* trees

17   suggests instead that it is an early offshoot from the common ancestor of all *Ictiobus* species.

18   This result is consistent with its morphology, which differs considerably from that of other

19   *Ictiobus* (Meek, 1904, personal observation). Moreover, the species inhabits clear, fast-flowing

20   streams in upland portions of the Río Pánuco system (Miller, in review; personal observation), a

21   habitat unlike that of any of the other *Ictiobus* species. *Ictiobus bubalus* occurs in middle and

22   lower portions of the Río Pánuco system, but there is no evidence of hybridization between these

23   species.

1   For reasons alluded to above, we regard tree topologies based on MP analysis without

2   3rd position transitions and ML (results of which are virtually identical) as best for interpreting

3   catostomid interrelationships. In both of these trees, ictiobines are basal and sister to all other

4   catostomids, and cycleptines cluster with catostomines (Moxostomini), albeit as a paraphyletic

5   group. This result is most similar to the topology obtained by Smith (1992). Harris and

6   Mayden's (2001) "prefered" topology (that supported by both LSU and combined LSU and SSU

7   mitochondrial rDNA data) has *Myxocyprinus asiaticus* as the most basal catostomid and

8   ictiobines sister to *Cycleptus* plus Catostominae. Constraining our *cyt b* data to fit this pattern

9   does not significantly increase tree length (Table 2). *Myxocyprinus* is clearly divergent from

10   *Cycleptus* in both morphology and mtDNA sequence, and thus is probably best not regarded as a

11   member of subfamily Cycleptinae, as argued by Harris and Mayden (2001).

12   The basal position of ictiobines in our *cyt b* trees and in Smith's (1992) total evidence

13   phylogeny is consistent with the fossil record. Ictiobines (†*Amyzon* spp.) are known as fossils

14   dating back to the Paleocene of North America (Wilson, 1980) and the Eocene of Asia (Chang et

15   al., 2001). The earliest arguable *Cycleptus* fossil evidence is from the Oligocene of Montana.

16   Reports of *Cycleptus* and *Myxocyprinus* fossils from Asia are based on misidentifications

17   (Cavender, 1986; Chen et al., 2001). The earliest catostomine fossil evidence is from the middle

18   Miocene of North America (Cavender 1986). Thus, our results support the contention of Smith

19   (1992) that the trend in catostomid evolution was from the large, deep bodied, fishes with long

20   dorsal fins and small numbers of large scales, toward the smaller, more terete-bodied, small-

21   scaled, short dorsal-finned catostomine body plan.

22   Our *cyt b* sequence results further suggest that catostomids are more similar to minnows

23   than cobitids. Harris and Mayden's, (2001) found catostomids to be more closely related to

1   gyrinocheilids and cobitids (cobitoids) than cyprinids, a result generally consistent with an

2   unpublished, morphology-based, phylogenetic hypotheses of cypriniform relationships (Siebert,

3   1987). Harris and Mayden (2001) present data on genetic distances between cyprinids and

4   catostomids, but no data on genetic distances among catostomids, gyrinocheilids and cobitids for

5   comparison. The morphology of ictiobines is similar to that of Asian cyprinines, but differs

6   greatly from that of cobitoids. If the hypothesis that ictiobines are the most ancestral

7   catostomids is accepted, a sister relationship between catostomids and cyprinids requires fewer

8   morphological reversals, than the hypothesis that catostomids are sister to cobitoids.

9

1   **Literature Cited**

2   AVISE, J. C. 1994. Molecular markers, natural history, and evolution. Chapman and Hall, New

3   York.

4   BROILAY, J. N. GALTIER, R.M. BRITO, AND Y. BOUVET. 1998. Molecular phylogeny of

5   Cyprinidae inferred from cytochrome b DNA sequences. Molecular Phylogenetics and

6   Evolution 9:100-108.

7   CAVENDER, T. M. 1986. Review of fossil history of North American freshwater fishes. pp 699

8   -725, *In:* C.H. Hocutt and E.O. Wiley (Eds.) The Zoogeography of North American

9   Freshwater Fishes. Wiley InterScience, New York, 866 p.

10   CHANG, M., D. MIAO, Y. CHEN, J. ZHOU, AND P. CHEN. 2001. Suckers (Fish, Catostomidae)

11   from the Eocene of China account for the family's current disjunct distribution. Science

12   in China (series D 44:577-586.

13   CHANG, Y.S. F.L. HUANG, AND T.B. LO. 1994. The complete nucleotide sequence and gene

14   organization of carp (*Cyprinus carpio*) mitochondrial genome. J. Mol. Evol. 38:138-155.

15   DOWLING, T. E., AND G. J. P. NAYLOR. 1997. Evolutionary relationships of minnows in the

16   genus *Luxilus* (Teleostei: Cyprinidae) as determined from cytochrome b

17   sequences. Copeia 1997:758-765.

18   EITNER, B., C. KIMBRELL, AND R. VETTER. 1999. *Sebastes moseri* (Scorpaeniformes:

19   Scorpaenidae): A new rockfish from the Eastern North Pacific. Copeia 1999: 85-

20   92.

21   ETNIER, D.A., AND W.C. STARNES 1993. The fishes of Tennessee. University of Tennessee

22   Press, Knoxville. 681 p.

23   FROST, D. R., H. M. CRAFTS, L. A .FITZGERALD, AND T. A. TITUS. 1998. Geographic variation,

1   species recognition, and molecualr evolution of cytochrome oxidase I in the *Tropidurus*

2   *spinulosus* complex (Iguania: Tropiduridae). Copeia 1999: 839-865.

3   HARRIS, P.M., AND R.L. MAYDEN. 2001. Phylogenetic reslationships of major clades of

4   Catostomidae (Teleostei: Cypriniformes) as inferred from mitochondrial SSU and LSU

5   rDNA sequences. Molecular Phylogenetics and Evolution. 20:225-237.

6   HILLIS, D.M., B.K. MABLE, A. LARSON, S.K. DAVIS, AND E.A. ZIMMER. 1996. Nucleic Acids

7   IV: Sequencing and cloning. pp 321-378, *In:* D.M. Hillis, C. Moritz, and B.K. Mable

8   (Eds.) Molecular Systematics, Second Edition. Sinauer Associates, Sunderland, MA.

9   HUBBS, C. L. 1930. Materials for a revision of the catostomid fishes of eastern North America.

10   Misc. Publ. Mus. Zool. Univ. Michigan 20: 1-47.

11   HUELSENBECK, J.P., AND F.R. RONQUIST. MRBAYES: Bayesian inference of phylogeny.

12   Biometrics (*in press*).

13   JOHNSON, D.W., AND W.L. MINCKLEY. 1969. Natural hybridization in buffalofishes, genus

14   *Ictiobus*. Copeia 1969:198-200.

15   KOCHER, T. D., AND C. A. STEPIEN. 1997. Molecular systematics of fishes. Academic Press,

16   San Diego, CA.

17   _____, AND K. L. CARLETON. 1997. Base substitution in Fish Mitochondrial DNA: patterns

18   and rates, pp 13-24. *In:* T. D. Kocher and C. A. Stepien (eds) , Molecular systematics of

19   fishes, Academic Press, San Diego, CA.

20   MAYDEN, R.L., B.M. BURR, L.M. PAGE, AND R.R. MILLER 1992. Names of North American

21   Freshwater Fishes, pp. 827-863. *In:* R.L. Mayden (ed.) Systematics and historical

22   ecology of North American freshwater fishes. Stanford University Press.

23   LYDEARD, C., AND K. J. ROE. 1997. The phylogenetic utility of the mitochondrial cytochrome b

1   gene for inferring relationships among Actinopterygian fishes, pp 185-303. *In:* Molecular

2   systematics of fishes. T. D. Kocher and C. A. Stepien (eds). Academic Press. San Diego,

3   CA.

4   MEEK, S. E. 1904. The fresh-water fishes of Mexico north of the Isthmus of Tehuantepec.

5   Field

6   Columbian Mus. Publ. 93 (Zool.) 5: ix-xix, 1-252.

7   METTEE, M.F., Jr. P.E. O'NIEL, AND J.M. PIERSON. 1996. Fishes of Alabama. Oxmoor House.,

8   Birmingham, AL.

9   MILLER, R.M. Fishes of Mexico. University of Chicago Press (*in review*).

10   MURAKAMI, M., Y. YAMASHITA, AND H. FUJITANI. 1998. The complete sequence of

11   mitochondrial genome from a gynogenetic triploid 'ginbuna' (*Carassius auratus*

12   *langsdorfi*) Zool. Sci. 15:335-337.

13   PERDICES, A., AND I. DOADRIO. 2001. The molecular systematics and biogeography of the

14   European cobitids based on mitochondrial DNA sequences. Molecular Phylogenetics and

15   Evolution. 19:468-478.

16   PERRY, W.G. Black and bigmouth buffalo spawn in brackish water ponds. 1976. Progressive

17   Fish Culturist 38:81.

18   POSADA, D., AND CRANDALL. K.A. 1998. Modeltest: testing the model of DNA substitution.

19   Bioinformatics 14(9): 817-818.

20   ROBISON H.W., AND T.M. BUCHANAN. 1988. Fishes of Arkansas. University of Arkansas Press.

21   Fayetteville, 536 p.

22   SHIMODAIRA, H. AND M. HASEGAWA. 1999. Multiple comparisons of log-likelihoods with

23   applications to phylogenetics. Mol. Biol. Evol. 16:1114-1116.

1 SMITH, C.L. 1962. Some Pliocene fishes from Kansas, Oklahoma, and Nebraska. Copeia

2   1962:505-520.

3 SMITH, G.R. 1981. Late Cenozoic freshwater fishes of North America. Ann. Rev. Ecol. Syst.

4   12:163-193.

5 _____.1992. Phylogeny and biogeography of the Catostomidae, freshwater fishes of North

6   America and Asia, pp 778-826, *In:* R.L. Mayden (ed.) Systematics and historical

7 ecology   of North American freshwater fishes. Stanford University Press.

8 _____, AND J.G. LUNDBERG. 1972. The Sand Draw fish fauna. pp. 40-54, *In:* M.F. Skinner

9   and C.W. Hubbard (Eds.) Early Pleistocene preglacial and glacial rocks and faunas of

10   north-central Nebraska. Bulletin American Mus. of Nat. Hist. 148:1-148.

11 STEVENSON, J.H. 1964. Fish farming experiment station. U.S. Fish and Wildlife Service Circ.

12   179:79-100.

13 SUTTKUS, R.D. AND H.L. BART, Jr. 2002 A preliminary analysis of the river carpsucker,

14   *Carpiodes carpio*, in the southern portion of its range, pp 209-221. *In:* L. Lozano (ed.)

15   Libro Jubilar en Honor al Dr. Salvador Contreras Balderas. Universidad Autonoma de

16   Neuvo Leon, Monterrey.

17 TAMURA, K., AND M. NEI.1993. Estimation of the number of nucleotide substitutions in the

18   control region of mirtochondrial DNA in humans and chimpanzees. Molecular Biology

19   and Evolution 10(3):512-526.

20 TANKA-UEUNO, T., M. MATUSI, G-F WU, L. FEI, AND O. TAKENAKA. 1999. Identity of *Rana*

21   *chensinensis* from other brown frogs as assessed by mitochondrial cytochrome-b

22   sequences. Copeia 1999: 187-190.

23 TEMPLETON, A.R. 1983. Phylogenetic inference from restriction endonuclease cleavage split

1   maps with particular reference to humans and apes. Evolution 37:221-244.

2   UYENO, T., AND G.R. SMITH 1972. Tetraploid origin of the karyotype of catostomid fishes.

3   Science 175:644-646.

4   WILSON, M.V.H. 1980. Oldest known *Esox* (Pisces: Esocidae) part of a new Paleocene teleost

5   fauna from western Canada. Can. J. Earth Sci. 1783:307-312.

6   XIAO, W., Y. ZHANG AND H. LIU. 2001. Molecular systematics of Xenocyprinae (Teleostei:

7   Cyprinidae): taxonomy, biogeography and coevolution of a special group restricted in

8   east Asia. Mol. Phylogenet. Evol. 18:163-173.

Table 1. Species/populations of ictiobines and outgroups for which complete *Cyt-b* sequence

data were obtained.

_____

_____

Species	Population/ GenBank Accession.#	Reference

_____

Ingroup

Carpiodes carpio	Wisconsin R., Upper Miss. R. Dr., WI	
C. carpio	Kentucy R., Ohio R. Dr., KY	
C. carpio	Licking R., Ohio R. Dr., KY	
C. carpio	Sunflower R., Lower Miss. R. Dr., MS	
C. cyprinus	James River, Ches.Bay Dr., VA	
C. cyprinus	Kentucky R., Ohio R. Dr., KY	
C. cyprinus	Wisconsin R., Upper Miss. R. Dr., WI	
C. cyprinus (*"forbesi"*?)	Wisconsin R., Upper Miss. R. Dr., WI	
C. velifer	Kentucky R., Ohio R. Dr., KY	
C. velifer	Wisconsin R., Upper Miss. R. Dr., WI	
Ictiobus bubalus	Wisconsin R., Upper Miss. R. Dr., WI	
I. bubalus	Amite R., Lake Pont. Dr., LA	
I. bubalus	Río San Fernando, NL, MX	
I. cyprinellus	Wisconsin R., Upper Miss. R. Dr., WI	
I. cyprinellus	Green River, Ohio R. Dr., KY	
I. cyprinellus	Amite R., Lake Pont. Dr., LA	

Table 1. *continued*

Species	Population/ GenBank Accession.#	Reference
I. labiosus	Río Pánuco, Tamaul., MX	
I. "meridionalis"	Río Usumacinta, Chiapas, MX	
I. niger	Wisconsin R., Upper Miss. R. Dr, WI	
I. niger	Kentucky R., Ohio R. Dr, KY	
Outgroups		
Cycleptus elongatus	Licking R., Ohio R. Dr., KY	
Moxostoma erythrurum	Eel R, Ohio R. Dr., IN	
M. hubbsi	St. Lawrence R, Quebec, CA	
M. valenciennesi	Eel R, Ohio R. Dr., IN	
Thoburnia hamiltoni	Mayo R, Roanoke R. Dr., VA	
Myxocyprinus asiaticus	Genbank AF036176	Xiao et al. 2001
Misgurnus anguillicaudatus	Genbank AF051868	Xiao and Zhang unpubl.
Cyprinus carpio	EMBL X61010	Chang et al. 1994
Carassius auratis	DDBJ NC002079	Murakami et al. 1998
Cobitis arachthosensis	Genbank AF263088	Perdices and Doadrio 2001

Table 2. Results of Templeton's (1983) and Shimodoira and Hasegawa's (1999) topological tests, comparing

alternate tree topologies for ictiobines and outgroups, as discussed in the text.   MP and ML trees are compared to

each other. Other trees compared to MP and ML trees. Only P values reported for Templeton and Shimodoira-

Hasegawa tests (** denotes significance at <0.01 level).

_____
_____

Topology	# trees	Tree length	-ln likelihood	Templeton test	S-H test
Best MP Tree	16	1249	6607.35	1.000	0.437
Ictiobines basal and sister to all other catostomids (ML tree)	16	1250	6599.19	0.8886	1.000
Myxocyprinus basal, Ictiobines sister to *Cycleptus* plusMoxostomini	16	1254	6601.54	0.5287	0.758
Carpiodes species monophyletic	48	1259	6648.05	0.0039**	0.002**
Ictiobus species monophyletic	4	1251	6616.25	0.1573	0.149

FIGURE LEGENDS

Figure 1. Phylogenetic relationships of ictiobines, cycleptines (*Myxocyprinus asiaticus* +

Cycleptus elongatus) and catostomines based on Smith's (1992) total evidence analysis.

Figure 2. Transitions (solid diamonds) and transversions (open diamonds) vs. p distance for 1st,

2nd and 3rd position substitutions based on pairwise comparisons of Cytochrome b sequence data

for ictiobines and outgroups.

Figure 3. Neighbor-joining tree depicting differences among ictiobines and other catostomids

(outgroups) as Tamura-Nei distances.

Figure 4. Phylogenetic relationships of ictiobines and outgroups based on unweighted maximum

parsimony analysis of complete Cytochrome b sequence data. Values beside the nodes represent

bootstrap support of 50% or greater (based on 1000 replicates).

Figure 5. Phylogenetic relationships of ictiobines and outgroups based on maximum likelihood

analysis of complete Cytochrome b sequence data, with branch support based Bayesian analysis.

†*Amyzon*

Ictiobus cyprinellus

Ictiobus labiosus

Ictiobus bubalus

Ictobus niger

Carpiodes cyprinus

Carpiodes velifer

Carpiodes carpio

Myxocyprinus asiaticus

Cycleptus elongatus

Catostominae

Cycleptus elongatus
Thoburnia hamiltoni
Moxostoma erythrurum
M. valenciennesi
M. poecilurum
Myxocyprinus asiaticus
Ictiobus labiosus
I. bubalus Río San Fernando
I. bubalus Upper Miss R.
I. bubalus Lake Pontchartrain Dr.
I. meridionalis Río Usumacinta
I. cyprinellus Upper Miss. R.
I. cyprinellus Ohio R.
I. niger Ohio R.
I. cyprinellus Lake Pontchartrain Dr.
I. niger Upper Miss. R.
C. cyprinus James River
C. carpio Ohio R.
C. cyprinus Ohio R.
C. carpio Upper Miss. R.
C. cyprinus Upper Miss. R.
C. "forbesi" Upper Miss. R.
C. velifer Ohio R.
C. velifer Upper Miss. R.
C. carpio Lower Miss. R.
C. carpio Ohio R.

0.1

A phylogenetic tree (cladogram) with bootstrap support values and the following terminal taxa:

- *C. cyprinus* James River
- *C. carpio* Lower Miss. R.
- *C. carpio* Ohio R.
- *C. velifer* Ohio R.
- *C. velifer* Upper Miss. R.
- *C. carpio* Upper Miss. R.
- *C. cyprinus* Upper Miss. R.
- *C. "forbesi"* Upper Miss. R.
- *C. carpio* Ohio R.
- *C. cyprinus* Ohio R.
- *I. labiosus* Río Pánuco
- *I. cyprinellus* Lake Pontchartrain Dr.
- *I. niger* Upper Miss. R.
- *I. niger* Ohio R.
- *I. bubalus* Upper Miss
- *I. bubalus* Lake Pontchartrain Dr.
- *I. bubalus* Río San Fernando
- *I. meridionalis* Río Usumacinta
- *I. cyprinellus* Upper Miss. R.
- *I. cyprinellus* Ohio R.
- *Myxocyprinus asiaticus*
- *Cycleptus elongatus*
- *Thoburnia hamiltoni*
- *Moxostoma erythrurum*
- *Moxostoma valenciennesi*
- *Moxostoma poecilurum*

Support values: 100, 70, 96, 54, 88, 100, 75, 95, 100, 100, 76, 68, 92, 100, 81, 100