Molecular Phylogenetics and Evolution 63 (2012) 159–167

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

A different perspective on the phylogenetic relationships of the Moxostomatini (Cypriniformes: Catostomidae) based on cytochrome-b and Growth Hormone intron sequences

Mark D. Clements [a,*], Henry L. Bart Jr. [a], David L. Hurley [b]

[a] Tulane University Museum of Natural History, Belle Chasse, LA 70037, USA
[b] East Tennessee State University College of Pharmacy, Department of Pharmaceutical Sciences, Johnson City, TN 37614, USA

ARTICLE INFO

Article history:
Received 25 April 2011
Revised 21 December 2011
Accepted 3 January 2012
Available online 12 January 2012

Keywords:
Phylogenetics
Growth Hormone
Introns
Cytochrome-b
Incongruence
Evolution

ABSTRACT

We have examined phylogenetic relationships of suckers of tribe Moxostomatini (Cypriniformes, Catostomidae) using cytochrome-b and Growth Hormone gene intron sequences. Phylogenies were significantly different from recent estimates of relationships based primarily on morphology (Smith, 1992) and cytochrome-b sequences (Harris et al., 2002). Overall, there was little support for many basal nodes in the phylogeny, however it was clear that *Scartomyzon* and *Moxostoma* were not monophyletic, despite morphological evidence provided Robins and Raney (1956, 1957), Jenkins (1970), and Smith (1992). Growth Hormone sequences provided good support for a monophyletic Western *Scartomyzon* lineage and thus suggested a single ancestral invasion of *Scartomyzon*-like fishes into drainages of Texas and Mexico. Phylogenetic relationships of Western *Scartomyzon* are structured geographically and do not conform well to current taxonomy.

1. Introduction

The Moxostomatini is a tribe of suckers (Catostomidae: Cypriniformes) endemic to temperate streams, rivers, and lakes of eastern North America; the range of the tribe extends east of the Rocky Mountains from the Hudson Bay Drainage to its southern limit in Pacific drainages of the Mexican Transvolcanic axis (Lee et al., 1980). The tribe includes redhorse suckers (*Moxostoma*), jumprock suckers (*Scartomyzon*), the spotted sucker (*Minytrema melanops*), chub suckers (*Erimyzon*), hog suckers (*Hypentelium*), torrent suckers (*Thoburnia*), and the extinct harelip sucker (*Lagochila lacera*) (Smith, 1992).

Most of the species diversity of tribe Moxostomatini is found in genera *Moxostoma* (ca. 12 spp.) and *Scartomyzon* (ca. 11 spp.). Hubbs (1930), Robins and Raney (1956, 1957), and Jenkins (1970) considered *Scartomyzon* to be a subgenus of *Moxostoma*, although several morphological characters and ecological preferences distinguish the two genera (Jenkins, 1970). The genus *Scartomyzon* was resurrected from synonymy of *Moxostoma* by Smith (1992) based on phylogenetic analysis of morphological, biochemical, and early life history characters, although Smith (1992) acknowledged, based upon alternative phylogenetic reconstructions, the potentially poly- or paraphyletic nature of the genera. Recently, Harris and Mayden (2001), Harris et al. (2002) and Doosey et al. (2010) found *Moxostoma* and *Scartomyzon* to be paraphyletic based on phylogenetic analysis of mitochondrial DNA sequences. Harris and Mayden (2001) and Harris et al. (2002) suggested subsuming *Scartomyzon* back into *Moxostoma*.

Based on the distribution of *Scartomyzon* it is not surprising that the genus could be paraphyletic. The highly disjunct distribution of this group suggests that eastern and western groups of *Scartomyzon* could have different ancestors among the Moxostomatini. The ecological and morphological similarities between eastern and western *Scartomyzon* could be due to convergence due to adaptive evolution to similar, upland habitats as suggested, but ultimately dismissed, by Robins and Raney (1957). However, Harris et al. (2002) found evidence for sister group relationships among some eastern and western *Scartomyzon* species, suggesting that these species share common ancestry. But, the disjunct species pairs recovered in Harris et al.'s (2002) genetic analysis differed from those proposed by Robins and Raney (1956, 1957) and Smith (1992), suggesting a disconnect between morphological and genetic characters that unite species pairs.

As noted by Smith (1992), the extremely disjunct distribution of *Scartomyzon* is one of the most peculiar among North American freshwater fishes. At present there is no widely accepted hypothesis to explain this pattern, although some rather unparsimonious

* Corresponding author. Present address: Department of Natural Sciences, Northern Essex Community College, 100 Elliot Street, Haverhill, MA 01830, USA. Fax: +1 978 556 3135.
E-mail address: mclements@necc.mass.edu (M.D. Clements).

1055-7903/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2012.01.001

biogeographic hypotheses have been proposed. Robins and Raney (1956) believed that the historical distribution of *Scartomyzon* was once continuous and extended from Georgia along the Gulf Slope into Texas. Intermediate populations, from Georgia to Texas, were thought to have been extirpated due to competition with an ecologically similar species, *Moxostoma poecilurum*. Jenkins (1970) hypothesized dispersal of ancestral *Scartomyzon* stock from the Atlantic slope, across the eastern continental divide into the upper Tennessee River system. Populations then dispersed down the Mississippi River into the Red River, then west and south, eventually reaching the Brazos River drainage in Texas, and finally south into Mexico. Like Robins and Raney (1956), Jenkins (1970) argued that intervening populations were extirpated due to competition, except that the competition involved ecologically similar upland darters (*Etheostoma* and *Percina*) of the central Mississippi basin.

The purpose of the present analysis is to further test competing phylogenetic and biogeographic hypotheses of moxostomatin suckers, and in particular *Scartomyzon*, using nuclear and mitochondrial gene sequences. This analysis expands the analyses of Smith (1992), Harris and Mayden (2001), Harris et al. (2002), and Doosey et al., 2010 by using specimens of all currently recognized species and as many populations as could be sampled within the ranges of currently recognized species and subspecies. The analysis also includes several recognized but undescribed taxa that were not included in Harris and Mayden (2001) or Harris et al. (2002).

2. Materials and methods

2.1. DNA extraction

Tissue samples were collected from specimens representing multiple populations of species and subspecies within tribe Moxostomatini (Table 1). When possible, individuals from at least two different populations were included for each taxon to represent intraspecific genetic variation and to help avoid errors in interpretation of results due to confounding factors such as introgression, ancestral polymorphisms, and PCR contamination.

For each specimen, approximately 200 mg of pectoral fin or muscle tissue was clipped from the right side and preserved in 70–95% ethanol. Smaller individuals were preserved whole in 95% ethanol. Prior to DNA extraction, 10–25 mg of fin or muscle tissue was rinsed with distilled water and blotted dry with a sterile Kimwipe® to remove residual ethanol. For some individuals, total DNAs were extracted using the Qiagen DNeasy tissue kit according to the manufacturer's protocol. Alternatively, DNAs were extracted by a modified Chelex-100/proteinase-K extraction protocol (Walsh et al., 1991). For Chelex® extractions, tissues were incubated for 2–12 h in a 5% solution (10 mM Tris–HCl) of Chelex® (BioRad) containing 20 μg proteinase-K. Extraction suspensions were then incubated for 15 min at 95 °C to denature enzymes and lyse cells to release DNA into solution. Crude extracts were then centrifuged for 10 min at 16,000*g* to pellet Chelex® resin and residual debris.

Table 1
Log likelihoods of optimal maximum likelihood trees and trees constrained to match alternative hypotheses, and results from AU topology tests (corrected *p*-values by Akaike weights averaging; values <5.0% indicate topologies that are significantly different from the best tree).

Topology	Cytochrome-b		GH	
	Log likelihood	*p*-Value	Log likelihood	*p*-Value
Optimal	−14360.883498	–	−4491.592459	–
Smith (1992)	−14962.729267	0.00	−4649.652294	0.00
Harris et al. (2002)	−14707.240782	0.11	−4584.398287	0.01
Moxostoma monophyletic	−14831.190035	0.41	−4531.116265	0.09
Scartomyzon monophyletic	−14832.635839	0.00	−4542.713929	0.00

2.2. PCR amplification and sequencing

The entire 1140 bp coding region of the cytochrome-b gene was PCR amplified using oligonucleotide primers GLU (5′-TAA CCG AGA CCA ATG ACT TG-3′) and THR (5′-ATC TTC GGA TTA CAA GAC CG-3′). GH intron sequences were amplified using novel oligonucleotide primers GHI3F (5′-TCT GCA ACT CTG ACT CCA TAG-3′) and GHI3R (5′-GAC GGT TCC ACT CAA GGT CTG-3′) (Clements, 2008). These primers amplified the 3rd intron of GH1 (i.e., Clements et al., 2004) in most catostomids. An additional novel downstream primer (GHI3R2 5-TGA GGC GGA AAG AGA TGTG AA-3′) was used in conjunction with GHI3F for amplification of this intron from some catostomid species. PCR reactions were performed in 25 μl total volumes containing 1.5 mM $MgCl_2$, 1× PCR buffer (Invitrogen), 0.2 mM each dNTP, 10 pmol of each primer, and 0.5 U *Taq* DNA polymerase (Invitrogen). PCR reactions were performed using 1–3 μl of Qiagen or Chelex® extracted DNA. After a 1 min denaturation at 94 °C, reactions were cycled according to the following temperature profile: 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 1:15 min, for 35 cycles. A final 10 min, 72 °C incubation was added at the end of 35 cycles to ensure complete extension of PCR products. PCR products were isolated and purified with the QIAquick PCR Purification Kit (Qiagen) or ExoSAP-IT (USB) and used as template for dye terminator cycle sequencing reactions.

In the present study, complications associated with phylogenetic analysis of paralogous sequences due to the tetraploid genome of catostomids (Uyeno and Smith, 1972) were avoided by using primers that preferentially amplify introns from GHI (Oakley and Phillips, 1999; Clements et al., 2004; Clements, 2008; Bart et al., 2010). Also, introns of GHI and GHII of catostomids are not easily confused at the sequence level. Clements (2008) and Bart et al. (2010) found significant length differences between paralogous copies of intron 3 making it trivial to isolate and sequence homologous introns. Furthermore, there is substantial sequence divergence (<60%) between introns from paralogous copies of catostomid GH's and thus paralogous sequences are unalignable outside of the first 20–30 bp adjacent to the intron splice junctions (Clements, 2008).

2.3. Phylogenetic analysis

Phylogenetic relationships were estimated with maximum likelihood and Markov Chain Monte Carlo based Bayesian inference. Maximum-likelihood analysis used the MPI version of RaXML v7.0.4 (Stamatakas, 2006) on the Odyssey cluster supported by the Harvard FAS Research Computing Group. The maximum likelihood tree was inferred using the rapid hill-climbing algorithm, starting from a parsimony tree, and support for nodes was assessed with the fast bootstrap method using 1000 non-parametric bootstrap replicates. Branches with less than 70% ML bootstrap support were considered weakly supported (Hillis and Bull, 1993; Erixon et al., 2003: Cummings et al., 2003). Bayesian inference used PHYCAS (www.phycs.org) with a GTR + gamma model, with four discrete rate categories. PHYCAS allows unresolved topologies (i.e., polytomious topologies) to be sampled during phylogenetic estimation, which can improve inferences for data sets characterized by shallow divergence or those with short periods between branching events (i.e., "star tree paradox", Lewis et al., 2005). The polytomy prior for PHYCAS was set to $\exp(1.0)(e \approx 2.71828)$, which means that topologies with k internal nodes will be favored $e \approx 2.71828$ times more a priori than tree topologies having $k + 1$ internal nodes. Thus, a more-resolved topology ($k + 1$) has to be

at least one log-likelihood unit better than a less-resolved topology (k) in order to overcome this prior (Lewis et al., 2005). Default priors and those suggested in software documentation were assumed for other model parameters. Two independent runs, seeded with different random starting parameters, were performed and PHYCAS was run for 100,000 cycles and the chain was sampled every 10 cycles. Less than optimal trees were discarded prior to production of a 95% majority-rule consensus.

Congruence between optimal trees inferred from cytochrome-b sequences and alternative topologies were assessed with approximately unbiased (AU) tests (Shimodaira and Hasegawa, 1999, 2008) using the scaleboot package in R. Backbone constraint topologies and site-wise likelihoods for AU tests were inferred with RaXML v7.0.4 (Stamatakas, 2006).

3. Results

3.1. Taxon sampling

Cytochrome-b sequence data were generated for a total of 105 (JF799431–JF799532) individuals representing 45 species of suckers, the largest such data set ever analyzed for this group. Sequences for outgroup taxa were retrieved from GENBANK and included *Cyprinus carpio* (NC_001606) and *Misgurnus fossilis* (AF263097). Functional outgroups sequenced for this analysis included a limited sampling of other groups of the family Catostomidae. The subfamily Ictiobinae is represented by *Ictiobus cyprinellus*, *I. bubalus*, *I. niger*, *I. labiosus*, *Carpiodes velifer, C. cyprinus* (James and Mississippi River), and *C. carpio*. The subfamily Cycleptinae is represented by *Cycleptus elongatus* and *Myxocyprinus asiaticus* (AF036176), the sequence for the latter retrieved from GENBANK. Within subfamily Catostominae, six taxa were sequenced for the tribe Catostomini, including three populations of *Catostomus commersonii, C. conchos, C. nebuliferus*, and *C.* sp. *cf. nebuliferus* (Rio Mezquital).

Tribe Erimyzonini (*sensu* Hubbs, 1930) is represented by three populations of *Minytrema melanops*. Tribe Thoburniini (*sensu* Hubbs, 1930) is represented by one specimen of each of the nominal forms. Tribe Moxostomatini included sequences of all described taxa and recognized but undescribed forms (e.g., Carolina redhorse). Multiple individuals were included for all included species except *Scartomyzon lachneri*, *S. ariommus, S.* sp. *cf. albidus* (Rio Soto la Marina), Carolina redhorse, sicklefin redhorse, and *Moxostoma valenciennesi*. Tissues of *M. pisolabrum* were also not collected or available for this analysis. The closest representative of this questionable species is from a geographically close population of *M. macrolepidotum* in the upper Mississippi River. Despite two collecting trips to the Rio Mezquital system, we were unable to obtain specimens of *S. milleri*. The extinct *L. lacera* was also not included.

A total of 39 catostomid taxa (JF799533–JF799571) were sequenced for Growth Hormone I Intron 3 (Clements et al., 2004). In addition to taxa that were unavailable or proved to be problematic to amplify and sequence cytochrome-b, GH introns could not be directly sequenced for *Moxostoma collapsum* and *Hypentelium etowanum*. Sequences for the latter species were found to be heterozygous with 2 or more indels resulting in uneditable electropherograms. The Intron 3 sequence of *Ictiobus bubalus* from the Amite River (Lake Pontchartrain Basin) was chosen as the outgroup for GH sequence analysis because introns of the GH gene are unalignable outside the family Catostomidae (Clements and Bart, unpublished data). Two species were sequenced to represent the Catostomini (*C. commersonii* and *C. conchos*) and the Erimyzonini was represented by only *Minytrema melanops*; the remaining taxa are formally assigned to tribe Moxostomatini or the closely related tribe Thoburniini. Taxon sampling was not as exhaustive as in the cytochrome-b dataset because preliminary analysis revealed very little intraspecific sequence variation for GH introns.

3.2. Sequence variation

The alignment of cytochrome-b sequences for the 105 specimens contained 444 parsimony informative characters with 365 of these in the 3rd codon position, 13 in the 2nd codon position, and 66 in the 1st codon positions. An examination of transitions and transversions vs. sequence divergence for each codon position revealed that 3rd codon position transitions show evidence of saturation above ~8% sequence divergence. Mean, pairwise within-group sequence divergences ranged ~6–10% among species complexes of tribe Moxostomatini.

GH sequences of the 39 taxa varied in length from 618 bp to 1218 bp. The sequences included the complete sequence of Intron 3 flanked by 4 bp of Exon 2 and 25 bp of Exon 3. The Catostomini had the shortest sequences (618–626) and *Minytrema melanops* (1218 bp) the longest. Among the Moxostomatini of Smith (1992), sequences ranged 859–1176 bp. *M. erythrurum* and *M. carinatum* had comparatively short sequences (859–862 bp) whereas *M. robustum* had very long GH intron sequences (1171–1176 bp). Remaining moxostomatin sequences ranged from 930 bp to 980 bp with an average length of 958 bp.

The final GH sequence data matrix consisted of 1531 bp (including indels) with 292 variable characters and 106 parsimony informative characters. Pairwise GH sequence divergences among major groups were less than those found for cytochrome-b. Outgroup and functional outgroup (Catostomini and *Minytrema*) divergences ranged 5.2–10.6%. Ingroup divergences were much lower, ranging from 1.4% to 4.4%. Within genus *Moxostoma*, sequence divergences were <3.4% and similar to divergences within the genus *Scartomyzon*. Sequence divergences within the Thoburniini were comparatively higher, ranging from 0.2% to 5.0%. GH sequences showed no evidence of saturation in either transitions or transversions.

3.3. Phylogenetic analysis

The results of Maximum Likelihood (ML) and Bayesian analysis (BA) of cytochrome-b sequence variation for 103 catostomid taxa are shown in Fig. 1. The family Catostomidae is monophyletic supported by 98% bootstrap (BS) and >95% bayesian posterior probability (BPP). The subfamily Catostominae is also monophyletic (98% BS, >95% BPP) with the Erimyzonini (100% BS, >95% BPPP) placed sister to (96% BS, >95% BPP) a monophyletic (100%, >95% BPP) Catostomini. The Catostomini + Erimyzonini clade was sister to a monophyletic (100%, >95% BPP) Moxostomatini (*sensu* Jenkins, 1970).

The analysis does not recover *Thoburnia* as monophyletic or unite genera *Thoburnia* and *Hypentelium* in a monophyletic Thoburniini (*sensu* Harris et al., 2002). Instead, *Thoburnia atripinnis* was placed in a clade (80% BS, >95% BPP) with *Hypentelium* species. *Hypentelium roanokense* was supported as sister (83% BS) to *H. nigricans* and *H. etowanum* in only ML analysis.

Relationships within and among remaining members of the Moxostomatini are mostly unresolved and there is no BS or BPP support for the monophyly of either *Scartomyzon* or *Moxostoma*. Eastern forms of *Scartomyzon* have affinities with different groups of moxostomatin suckers, or are unresolved. However, Western *Scartomyzon* are part of a well-supported clade (99% BS, >95% BPP) that includes *M. valenciennesi*, *M. poecilurum*, *M* sp. "Apalachicola redhorse", and *M. duquesnei*. There is a well-supported clade comprising *M. poecilurum* and *S. congestus* populations (98% BS, >95% BPP). In ML analysis, *M. duquesnei* is found to be the sister-group to the latter clade (86% BS). There is also strong evidence

Fig. 1. Phylogenetic relationships of the Moxostomatini and functional outgroups estimated from c cytochrome-b sequences. The maximum likelihood topology is shown and numbers above nodes are bootstrap support values ⩾70%. Thickened branches indicate Bayesian posterior support values ⩾95%.

for geographical structure within *S. congestus,* with populations from the Colorado, Brazos, and Rio Bravo drainages forming a well supported clade (97%, >95% BPP) separate from populations from the San Antonio (type locality of *S. congestus*) and Nueces drainages (100%, >95% BPP).

Remaining members of western *Scartomyzon* also form a well-supported clade (100% BS, >95% BPP). An undescribed form of *S. austrinus* from the Rio Conchos is sister to a well-supported clade (100% BS, >95% BPP) containing *S. albidus*, *S. austrinus*, *S. mascotae*, and an undescribed form of *S. austrinus* from the tributaries of the Rio Grande de Santiago drainage. This analysis suggests that populations of *S. albidus* from the Rio Soto la Marina are distinct from other *S. albidus* populations and likely represent a new taxon since they are not resolved as part of the well-supported clade (100% BS, >95% BPP) containing other *S. albidus*. Like *Scartomyzon* from Texas, there is evidence for geographic structure within *S. albidus*. Populations from Gulf of Mexico drainages south of the Rio Bravo (i.e., Rio San Fernando) form a well-supported clade (100% BS, >95% BPP) separate from populations from tributaries of the Rio Bravo (96% BS, >95% BPP). The remaining members of western *Scartomyzon* are all found in Pacific drainages of Mexico and form a group with high support (95% BS, >95% BPP). Within this Pacific group, the undescribed species from tributaries of the Rio Grande de Santiago are sister to a clade composed of populations of *S. austrinus* (including Rio Lerma = type locality population) and the endemic *S. mascotae*.

Similar to Eastern *Scartomyzon*, the affinities among most species of *Moxostoma* were not resolved well by ML or BA. The species groups that were recovered in our analysis include; (1) a *Moxostoma* sp. "sicklefin redhorse" + *M. carinatum* clade (100% BS, >95% BPP), (2) a shorthead redhorse clade, which includes *M. macrolepidotum* and *M. breviceps*, and *M. hubbsi* as the sister species to this group (100% BS, >95% BPP); (3) a *M. collapsum* + *M. anisurum* clade (100%, >95% BPP) that was placed (81% BP, >95% BPP) with *M. pappillosum*, *M.* sp. "Carolina redhorse", and *S. ariommus*.

The results of ML and BA analyses of GH sequence for 39 catostomid taxa are shown in Fig. 2. The Erimyzonini, represented by only *Minytrema*, is sister (85% BS only) to a monophyletic (100% BS, >95% BPP) Catostomini. This clade is in turn sister to a monophyletic (100% BS and >95% BPP) Moxostomatini (*sensu* Jenkins, 1970).

The Thoburniini (*sensu* Harris et al., 2002) is monophyletic (81% BS, >95% BPP) and sister to a more inclusive Moxostomatini (98% BS and >95% BPP). Within the Thoburniini, *Thoburnia* is paraphyletic with *T. atripinnis* basal (98% and >95% BPP) to a monophyletic *Hypentelium* clade (86%, >95% BPP). Remaining species of *Thoburnia* were placed in a well-supported clade that received 100% BS and >95% BPP support.

Relationships among the remaining members of the Moxostomatini are mostly unresolved and *Scartomyzon* and *Moxostoma* are not monophyletic. Among eastern forms of *Scartomyzon*, *S. lachneri* is sister to *M* sp. "Apalachicola redhorse" (89% BS, >95% BPP). Western *Scartomyzon* are monophyletic, forming a clade that is supported with 75% BS and >95% BPP and *S. congestus* is sister (>95% BPP only) to the remaining species in this Western clade. The relationships among remaining species of *Moxostoma* are also largely unresolved, although *M. carinatum* was placed (100% BS, >95% BPP) with *M. erythrurum* populations.

Comparison of maximum likelihood hypotheses to alternative hypotheses—Comparison of optimal and constraint topologies using AU tests (Table 1) revealed strong support for the optimal topologies recovered for each data set. The relationships proposed by Smith (1992) and Harris et al. (2002) are statistically different from relationships recovered in the current analyses. More generally, alternative topologies that constrained *Moxostoma* and *Scartomyzon* to be monophyletic were also statistically different from topologies recovered with cytochrome-b and GH introns sequences.

4. Discussion

The current analyses found good support for a monophyletic Catostominae. Most of the topologies placed *Minytrema* with the Catostomini (*sensu* Harris et al., 2002) as opposed to the Moxostomatini (*sensu* Smith, 1992). Recently, Doosey et al. (2010) found the Erimyzonini to be the earliest diverging lineage within the Catostominae and sister to remaining members of the Moxostomatini + Catostomini. A basal position for the Erimyzonini was first proposed by Miller (1959) based on osteological evidence and later recovered by Ferris and Whitt (1978) based on loss of duplicate (i.e., paralogous) gene expression patterns. Given the unstable position of this taxon across analyses, the evidence for the inclusion of this taxon in either the Catostomini (Harris et al., 2002), the Moxostomatini (Smith, 1992), or as the sister group to the Catostominae (Miller, 1959; Doosey et al., 2010) deserves further consideration.

All analyses support the recognition of the Thoburniini (*sensu* Harris and Mayden, 2001; Doosey et al., 2010) as the sister group to the remaining moxostomatin species. However, within this clade *Thoburnia* appears to be paraphyletic since *T. atripinnis* was always recovered as the sister group to *Hypentelium* rather than *Thoburnia* (Harris et al., 2002; Doosey et al., 2010). Nelson (1948, 1949) was first to recognize the affinities of *Thoburnia* and *Hypentelium*. Robins and Raney (1956), Bailey (1959), Jenkins (1970), and Smith (1992) considered *T. atripinnis* to be a close relative of *S. ariommus*. All molecular analyses to date suggest that *T. atripinnis* is intimately related to *Hypentelium,* as opposed to *S. ariommus* or other *Thoburnia* species. Thus, despite morphological evidence for the inclusion of *T. atripinnis* within *Thoburnia* (Bailey, 1959; Jenkins, 1970), molecular analyses suggest inclusion of this taxon in *Hypentelium*. Thus, the classification of *T. atripinnis* deserves reexamination.

None of the present analyses supported a monophyletic *Scartomyzon* as suggested also by Harris and Mayden (2001), Harris et al. (2002), and Doosey et al. (2010). Topological comparisons in the present study also strongly rejected a monophyletic *Scartomyzon* (Table 1). Considering the weight of evidence presented here, and in other molecular analyses (e.g., Harris and Mayden, 2001; Harris et al., 2002; Doosey et al., 2010), there is little molecular phylogenetic support for recognizing genus *Scartomyzon* as defined by Smith (1992), Jenkins (1970), and Robins and Raney (1956, 1957).

The cytochrome-b data set suggests independent evolutionary lineages of *Scartomyzon*-like species in Texas and Mexico (Fig. 1). However, Growth Hormone intron data provide strong support for a monophyletic Western *Scartomyzon* lineage (Fig. 2). A monophyletic Western *Scartomyzon* lineage is consistent with biogeography since this relationship suggests that all Western *Scartomyzon* share the same ancestor, a result consistent with the distribution of several other fish species complexes (e.g., *Campostoma anomalum*, *Cyprinella lutrensis*, *Ictalurus punctatus*, *Lepomis megalotis*, *Etheostoma* (*Oligocephalus*) *grahami*/*lepidum*) endemic to Texas and Mexico. Western representatives of these other fish groups are each presumed to have evolved by peripheral isolation from Mississippi River basin ancestors. Furthermore, a monophyletic Western *Scartomyzon* clade is supported by 4 synapomorphic substitutions in the GH matrix.

Eastern *Scartomyzon* species were not monophyletic in analyses based either on cytochrome-b or GH intron data. GH intron data recovered a sister group relationship between *S. lachneri* and *M.* sp. "Apalachicola redhorse". *Scartomyzon lachneri* has traditionally been associated with other members of the Eastern *Scartomyzon*

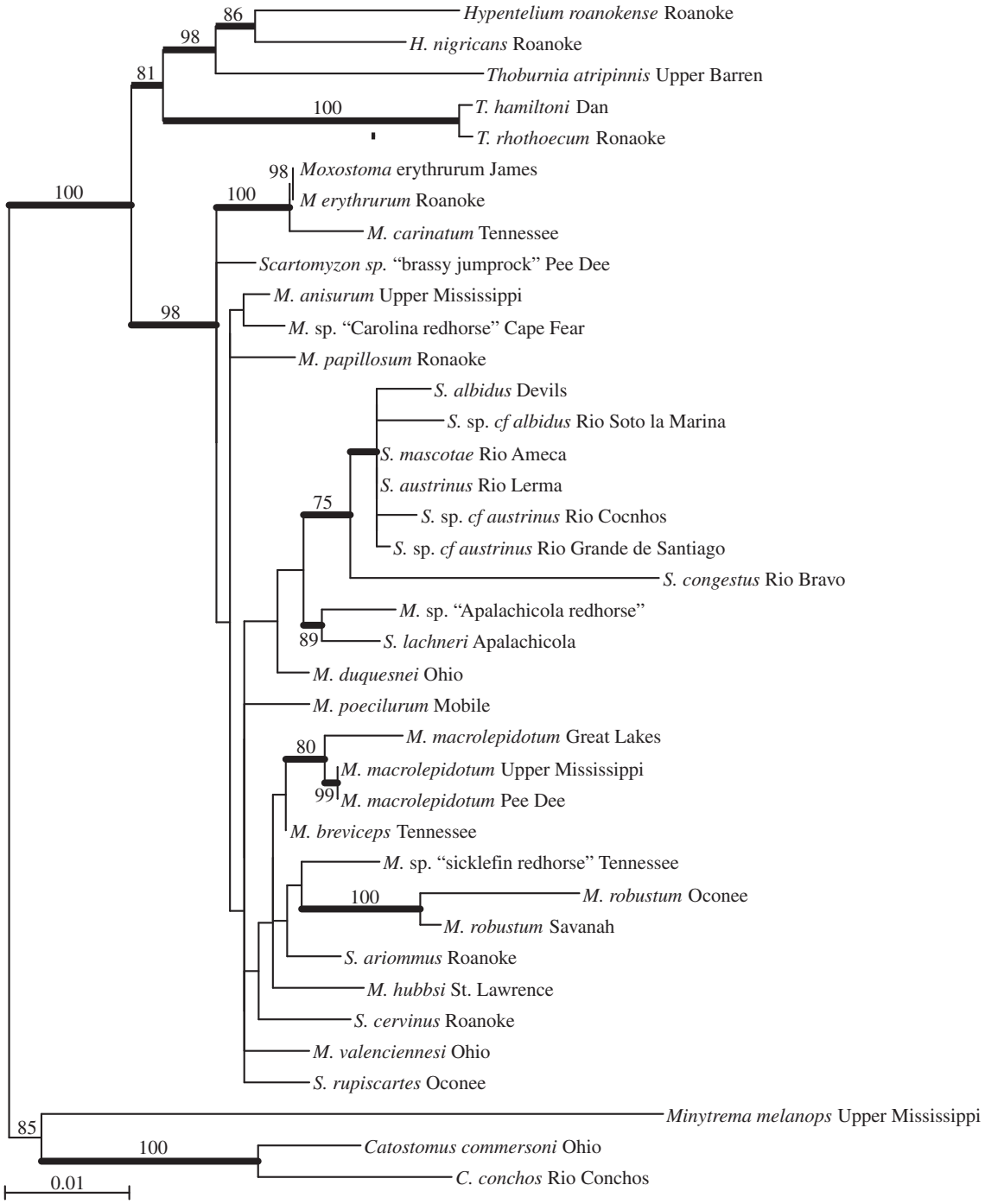

Fig. 2. Phylogenetic relationships of the Moxostomatini and functional outgroups estimated with Growth Hormone intron sequences. The maximum likelihood topology is shown and numbers above nodes are bootstrap support values ⩾70%. Thickened branches indicate Bayesian posterior support values ⩾95%.

lineage. In particular, Robins and Raney (1956, 1957) thought that *S. lachneri* shared a most recent common ancestor with *S. rupiscartes*. This relationship was also suggested by the analysis of Smith (1992). Buth (1978), on the other hand, found that *S. cervinus* and *S. lachneri* were most closely related. Jenkins (1970) concluded that the affinities of *S. lachneri* were unknown, but that it clearly was a member of the Eastern *Scartomyzon* lineage.

Jenkins (1970) presented strong morphological evidence that *M.* sp. "Apalachicola redhorse" was a recently derived, peripheral isolate of *M. poecilurum* and that the two species were so closely related that they should be recognized as a species group separate from all other *Moxostoma* species. Jenkins (1970) cited 24 morphological, osteological, and meristic characters that link these two species and that distinguish them from other *Moxostoma* species. *Scartomyzon. lachneri* and *M.* sp. "Apalachicola redhorse" are endemic to and sympatric in upland portions of the Apalachicola drainage, suggesting that the sister relationship could possibly be due to introgression. Differences in GH intron sequences between these species (1.4%) are only slightly larger than the divergences between presumed allopatric sister species *M. poecilurum* and *M.* sp. "Apalachicola redhorse" (1.2%). *Scartomyzon lachneri* shows a similar degree of divergence from other Eastern *Scartomyzon*

(1.4–1.9%). Thus, recent introgression seems unlikely. There are two synapomorphies for this species pair in the GH sequence matrix, a single base pair transversion (i.e., G/T) substitution, and an indel at position 220. Analysis of additional individuals of *M.* sp. "Apalachicola redhorse" and *S. lachneri* may be needed to resolve affinities of these species to other *Moxostoma* vs. other eastern *Scartomyzon* group taxa.

Moxostoma (*sensu stricto*) was also not found to be monophyletic in the current analysis and was strongly rejected as monophyletic by topological tests (Table 1). However, there is evidence to recognize certain species groups. For example, in the cytochrome-b tree there is support for a close relationship between a *M. carinatum* and *M.* sp. "Sicklefin redhorse" (Harris et al., 2002; Doosey et al., 2010). GH intron phylogenies placed *M. carinatum* with *M. erythrurum,* a relationship recovered in the analysis of Smith (1992) and first hypothesized by Jenkins (1970). Also, there was strong evidence from cytochrome-b analyses for a shorthead redhorse group comprising *M. macrolepidotum* and *M. breviceps* with *M. hubbsi* as the sister species to this clade (Harris et al., 2002, Doosey et al., 2010). *Moxostoma hubbsi* and *M. valenciennesi* have traditionally been the only species placed in the subgenus *Megapharynx* (Robins and Raney, 1956; Jenkins 1970; Smith, 1992). Cytochrome-b analysis suggests that *M. valenciennesi* is related to western *Scartomyzon*, *M. duquesnei*, *M. poecilurum*, and *M.* sp. "Apalachicola redhorse".

Similar to Doosey et al. (2010), *S. ariommus* was found to be a close relative of species in the V-lip redhorse group (i.e., *M. collapsum*, *M. anisurum*, and *M. pappillosum*), which is unusual given that morphological evidence (Jenkins, 1970; Bailey, 1959; Robins and Raney, 1956) has never linked *S. ariommus* with any species of *Moxostoma. Moxostoma.* sp. "Carolina redhorse" was also recovered as a member of the V-lip + *S. ariommus* clade, however this undescribed taxon is believed to be closely related to *M. erythrurum* based on anatomy, coloration, and reproductive behavior (Jenkins, unpublished data).

Overall, the molecular analyses presented here are significantly different from the mostly morphology-based hypothesis of Smith (1992). Most surprising however, the current analysis, and in particular the cytochrome-b trees, are significantly different from the cytochrome-b hypothesis of Harris et al. (2002). Comparison of the cytochrome-b results in the present study, which are nearly identical to those of Doosey et al. (2010), to the results in Harris et al. (2002) reveals important differences even though these studies used the same mitochondrial locus. Most notably, the Harris et al. (2002) preferred topology recovered some eastern and western *Scartomyzon* as sister species and had a different placement for *S. ariommus*. Comparison of cytochrome-b sequences for specimens that differed the most in phylogenetic placement between the two studies suggests that Harris et al. (2002) may have mistakenly labeled some of their sequences.

Comparison of Harris et al.'s (2002) cytochrome-b sequences of *S. lachneri* and *S.* sp. *cf. austrinus* (Rio Juchipila, MEX) to cytochrome-b sequences for the same species in the current study suggest that the names of the sequences were mistakenly transposed in their analysis. The sequences in the present study differ by no more than three substitutions (<0.3%) from the sequences with transposed names of Harris et al. (2002). The two species are not easily confused at the sequence level, differing by approximately 9% in the present study. Comparison of sequence of *S. ariommus* from the present study to that of Harris et al. (2002) suggests that there is also an error with these sequences. The sequences differ by approximately 6% and most substitutions are clustered in the 5′ end of the sequence, which suggests that mistakes were made while editing sequences. To eliminate suspicion of the *S. ariommus* sequence used in the present study, the tissue was re-extracted and re-sequenced twice producing the same sequence as the original sequence. Unfortunately, no additional specimens of this hard-to-catch species were available to provide independent confirmation of this result.

With the exception of the above discrepancies, the results of Harris et al. (2002) are quite similar to the cytochrome-b results of the present study (Fig. 1). If the names assigned to Harris et al. (2002) sequences of *S. lachneri* and *S.* sp. *cf. austrinus* are transposed to agree with the present study, there is no evidence for a sister relationship between eastern and western *Scartomyzon* taxa in their topology, consistent with the results of the present study.

According to accepted taxonomy, Western *Scartomyzon* comprises two species complexes: *S. congestus* and *S. austrinus*. Within *S. congestus*, there are two recognized subspecies, *S. c. congestus* and *S. c. albidus*, which have long been considered intimately related and were thought to intergrade in areas where distributions overlap (Robins and Raney, 1956, 1957; Jenkins, 1970). The *S. austrinus* species complex includes: *S. a. austrinus, S. a. milleri*, *S. mascotae* (Robins and Raney, 1956, 1957; Jenkins, 1970), and two undescribed forms in the Rio Conchos and Rio Grande de Santiago (Jenkins, personal communication; present author's data). The validity of *M. mascotae* has been questioned by Buth (1978), and Jenkins (*in* Lee et al., 1980), and Smith (1992) who considered it conspecific with *S. austrinus*.

It is clear that the current, accepted taxonomy of the Western lineage is incongruent with genealogical relationships derived from phylogenetic analyses of cytochrome-b and GH intron sequence variation (Figs. 1 and 2). *Scartomyzon congestus* and *S. albidus* are not closely related genetically; they differ by more than 10% cytochrome-b sequence divergence and by more than 3% GH sequence divergence. The affinities of *S. albidus* are clearly with the *S. austrinus* clade. Thus, the genetic results suggest that morphological similarities between *S. albidus* and *S. congestus* (Robins and Raney, 1956, 1957; Jenkins, 1970) are due to convergence. Given the high genetic divergence between these taxa and the lack of any evidence of intergradation in the present study, we recommend that *S. albidus* be elevated to the rank of full species and recognized as having closer affinities to *S. austrinus* than *S. congestus.*

Scartomyzon albidus occurs in the Rio Grande and certain tributaries below Big Bend, and the range extends south along the Gulf Slope to the Rio Soto la Marina drainage. However, the genetic analysis revealed that the population in the Rio Soto la Marina is divergent from other *S. albidus* populations (more than 6% cytochrome-b and 0.9% GH intron). All the populations deserve careful morphological analysis. Field observations revealed that it differs markedly in body form and coloration from other *S. albidus* populations (personal observation). If recognized as distinct, this would represent the third endemic species (with *Notropis aguirrepequenoi* and *Xiphophorus xiphidium*) from the Rio Soto la Marina drainage (Smith and Miller, 1986; Miller et al., 2006).

Based on available genetic evidence, the distribution of *S. albidus* (*sensu stricto*) should be restricted to the lower Rio Grande and tributaries (i.e., Devils River and Rio Ramos) and to the upper Rio San Fernando system (i.e., Rio Madroño). There was limited genetic differentiation between samples from the Devils River (above and below Dolan falls) and the Rio San Juan (i.e., Rio Ramos). But isolated populations in the Rio San Fernando (type locality system) were moderately divergent (ca. 1.2% cytochrome-b divergence) from Rio Grande populations. This level of divergence suggests that the Rio San Fernando population of *S. albidus* has been isolated from the Rio Grande populations for some time. The populations do not appear to have diverged sufficiently – genetically or morphologically – to warrant recognition as separate species. The ichthyofauna of Rio San Fernando system is very similar to that of the Rio Bravo, with many shared species and no endemic taxa known to occur there (Smith and Miller, 1986; Miller et al., 2006).

The large sequence divergences (8.1–9.0% cytochrome-b and 0.3–0.9% GH intron) discovered for the undescribed Rio Conchos form of *S. austrinus* confirms the distinctiveness of this taxon, as indicated also by morphology by Jenkins (personal communication). However, the phylogenetic placement of this taxon suggests that it is not closely related to *S. austrinus*. Rather, it is the most basal member of the expanded *S. albidus*/*S. austrinus* clade (Fig. 1). The current analysis suggests the recognition of the Rio Conchos population as a distinct species worthy of taxonomic description. It is confined to the Rio Conchos, Alamito Creek in Texas, and the reach of the Rio Grande near the confluence of these two streams (Jenkins *in* Lee et al., 1980). The Rio Conchos is noted for its high endemism with ~7 endemic species known only from this system (Smith and Miller, 1986; Miller et al., 2006).

The distinctiveness and phylogenetic placement of *S. a. milleri* (endemic to the Rio Mezquital; Robins and Raney, 1956) is unknown since efforts to obtain specimens for genetic analysis were unsuccessful. However, the basal position of specimens from the Rio Grande de Santiago (i.e., Rio Juchipila and Rio Balaños) relative to the rest of the *S. austrinus* (type locality = Rio Lerma) + *S. mascotae* complex, confirms its distinctiveness (Robins and Raney, 1956, 1957). It is quite distinct genetically (3.2% cytochrome-b sequence divergence) from *S. austrinus* and *S. mascotae*.

As stated earlier, species-level distinctiveness of *S. mascotae* has been questioned (e.g., Buth, 1978 and Smith, 1992). The current analysis revealed that this form is indeed not as divergent genetically from *S. austrinus* (*sensu stricto*) (0.7% cytochrome-b and 0.0% GH sequence divergence) as might be expected for a named species. Furthermore, the phylogenetic analysis (Fig. 1) placed this taxon sister to the Rio Armeria population of *S. austrinus*. The analysis resolves these populations with Rio Lerma and Morelia populations of *S. austrinus* as reciprocally monophyletic with high nodal support, suggesting that *S. mascotae* is part of a broader *S. austrinus* clade.

The Rio Lerma population of *S. austrinus* contains a 16 bp GH intron indel that distinguishes it from all other sequences, including *S. mascotae*. However, recognizing the Rio Lerma population of *S. austrinus* as distinct renders other *S. austrinus* and *S. mascotae* populations paraphyletic. The individuals included in the analysis for nominate *S. austrinus* came from the type locality system (Rio Lerma) and the isolated, neighboring and endorheic Rio Morelia system. There was very little difference among sequences obtained from specimens from these localities (ca. 0.4% cytochrome-b divergence), suggesting that these systems have not been isolated for a long period of time. The ichthyofaunas of the Rio Lerma and the Rio Morelia are intimately related; only one endemic species (*Chirostoma charari*) is known from the Morelia system (Miller and Smith, 1986; Miller et al., 2006).

The populations of *S. congestus* included in the analysis are highly divergent (>11.0% cytochrome-b and >2.5 GH sequence divergence) from other members of the Western lineage. Within the *S. congestus* clade, there are two divergent lineages that differ by ca. 2% cytochrome-b sequence divergence. One lineage comprises the San Antonio and Nueces populations. Interestingly, the other lineage comprises the Brazos and Colorado river populations (easternmost Gulf Slope populations), and the Pecos River and Pinto Creek populations of the Rio Grande system, west of the Nueces River. The type locality of *S. congestus* is the San Antonio River. The pattern of divergence observed in this study suggests that *S. congestus* should encompass both the San Antonio and Nueces river populations. Thus, populations in the Brazos and Colorado River, and the Pecos River and middle Rio Grande could represent divergent populations worthy of taxonomic recognition.

"*Moxostoma austrinum* (*sensu lato*) shows about the greatest morphological variation of all *Moxostoma* species" (Jenkins *in* Lee et al., 1980). Given the complex geographic structure genetically determined within the Western lineage, a thorough assessment of morphological variation is warranted. In addition to the populations of *S. congestus* mentioned above, populations that deserve further close morphological scrutiny include the *S. albidus*-like form from the Rio Soto la Marina, *S. albidus* from the Rio Madroño, *S.* sp. *cf. austrinus* from the Rio Grande de Santiago, and *S.* sp. *cf. austrinus* from the Rio Conchos.

5. Conclusions

We examined the phylogenetic relationships of suckers of tribe Moxostomatini (Cypriniformes, Catostomidae) using mitochondrial cytochrome-b gene sequences and nuclear intron sequences of the Growth Hormone gene. This study includes the most complete taxon sampling to date and is the first for the group to reconstruct phylogenetic relationships with nuclear DNA (nDNA) sequences.

Differences in relationships recovered in this analysis and in Doosey et al. (2010), compared to the recent mtDNA sequence-based analyses of Harris et al. (2002), are attributed to the mislabeling of certain species. As these discrepancies are accounted for here in, the current and former genetic studies produce similar results. This conclusion mostly demonstrates the importance of replicating data sets and performing parallel and independent analyses in phylogenetic studies.

It is clear from all molecular analyses that genus *Scartomyzon* is not monophyletic, despite contrary morphological evidence provided by Robins and Raney (1956, 1957), Jenkins (1970), and Smith (1992). The only species of *Scartomyzon* recovered as monophyletic in the present study are found in the western clade. The type species of *Scartomyzon* (*Teretulus cervinus*) (Fowler, 1913) is not a member of this clade and given the results of the preset analysis, *Scartomyzon* can only be recognized as monotypic (i.e., containing only *Scartomyzon cervinus*). So, it is perhaps best to follow the suggestions of Harris and Mayden (2001) and Harris et al. (2002) and recognize *Scartomyzon* as junior synonym of *Moxostoma*.

Growth Hormone intron sequences provided good support for a monophyletic Western *Scartomyzon* lineage and thus suggested a single ancestral invasion into drainages of Texas and Mexico. Phylogenetic relationships of Western *Scartomyzon* taxa are structured geographically and do not conform well to current taxonomy. The analyses also revealed cryptic endemism within this clade. The conclusions drawn from the analyses of western *Scartomyzon* suggest that this group deserves major taxonomic revision and perhaps recognition as a distinct genus separate from *Moxostoma* and eastern species formerly assigned to *Scartomyzon* (see above).

Overall, there was little support for many basal nodes in phylogenies derived from mtDNA and nDNA sequences in this study as most trees were characterized by numerous, unsupported polytomies. Although most basal relationships within this clade are unresolved at this time, there was strong to moderate support for removing *Erimyzon* and *Minytrema* from the Moxostomatini and for recognizing the Thoburniini (*Hypentelium* + *Thoburnia*) as the sister clade to all other *Moxostoma* and *Scartomyzon* species. Also, nDNA strongly supports a monophyletic Western *Scartomyzon* clade. There was also evidence to recognize a V-lip species group (*M. anisurum* + *M. pappillosum* + *M. collapsum*) and a *M. carinatum* species group (*M. carinatum* + *M. erythrurum* + *M.* sp. "sicklefin redhorse") and a shorthead redhorse species group (*M. macrolepidotum* + *M. breviceps*). Future phylogenetic endeavors into this group should focus on developing additional genetic loci that are intermediate in variation to those used in this study to avoid saturation and insure a moderate to high level of interspecific variability. It would also be worthwhile to revaluate morphological characters used by Smith (1992) and to search for additional

characters that could be used to determine the limits of genera and species groups within the Moxostomatini.

Acknowledgements

The following individuals donated specimens, assisted with collections, or provided assistance with analyses: R. Blanton, C. Bell, S. Carlton, M. Cashner, S. Contreras, E.D. Cope, M. Doosey, P. Fransisco Garcia, R. Jenkins, M. Kronforst, L. Lazano, G. Looney, J. Lyons, C. Marshall, R. Mayden, S. McInitch, N. Mercado-Silva, P. Nelson, K. Piller, S. Quek, M. Raley, W. Starnes, A. Snyder, T. Turner, and P. Unmack. R. Jenkins and an anonymous reviewer provided suggestions to improve the manuscript. Portions of this research were supported by a National Science Foundation grant (DEB-0237013) awarded to H. Bart and D. Hurley.

References

Bailey, R.M., 1959. A New Catostomid Fish, *Moxostoma (Thoburnia) atripinne*, from the Green River Drainage, Kentucky and Tennessee, vol. 599. Occasional Papers of the Museum of Zoology University of Michigan, pp. 1–19.

Bart Jr., H.L., Reneau, P.C., Doosey, M.H., Bell, C.D., 2010. Evolutionary divergence of duplicate copies of the Growth Hormone gene in suckers (Actinopterygii: Catostomidae). International Journal of Molecular Sciences 11, 1090–1102.

Buth, D.G. 1978. Biochemical Systematics of the Moxostomatini. Ph.D. Dissertation, Urbana, IL: Univeristy of Illinois.

Clements, M.D., 2008. Molecular Systematics and Biogeography of the Moxostomatini (Cypriniformes: Catostomidae), with Special Reference to Taxa in Texas and Mexico. Ph.D. Dissertation: New Orleans, LA, Tulane University.

Clements, M.D., Bart, H.L., Hurley, D.L., 2004. Isolation and characterization of two distinct growth hormone cDNAs from the tetraploid smallmouth buffalofish (*Ictiobus bubalus*). General and Comparative Endocrinology 136 (3), 411–418.

Cummings, M.P., Handley, S.A., Myers, D.S., Reed, D.L., Rokas, A., Winka, K., 2003. Comparing bootstrap and posterior probability values in the four-taxon case. Systematic Biology 52 (4), 477–487.

Doosey, M.H., Bart, H.L., Saitoh, K., Miya, M., 2010. Phylogenetic relationships of catostomid fishes (Actinopterygii: Cypriniformes) based on mitochondrial ND4/ND5 gene sequences. Molecular Phylogenetics and Evolution 54 (3), 1028–1034.

Erixon, P., Svennblad, B., Britton, T., Oxelman, B., 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. Systematic Biology 52 (5), 665–673.

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

Fowler, H.W., 1913. Notes on catostomid fishes. Proceedings of the Academy of Natural Sciences Philadelphia 6558, 45–60.

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 (2), 225–237.

Harris, P.M., Mayden, R.L., Espinosa Perez, H.S., Leon, F.G., 2002. Phylogenetic relationships of *Moxostoma* and *Scartomyzon* (Catostomidae) based on mitochondrial cytochrome b sequence data. Journal of Fish Biology 61 (6), 1433–1452.

Hillis, D.M., Bull, J.J., 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42 (2), 182–193.

Hubbs, C., 1930. Materials for the Revision of the Catostomid Fishes of Eastern North America. Miscellaneous Publications of the Museum of Zoology University of Michigan, pp. 1–47.

Jenkins, R.E., 1970. Systematic Studies of the Catostomid Fish Tribe Moxostomatini. Ph.D. Dissertation, Ithaca, NY, Cornell University.

Lee, D.S., Gilbert, C.R., Hocutt, C.H., Jenkins, R.E., McAllister, D.E., Stauffer Jr., J.R., 1980. Atlas of North American Freshwater Fishes. North Carolina State Museum of Natural History.

Lewis, P.O., Holder, M.T., Holsinger, K.E., 2005. Polytomies and Bayesian phylogenetic inference. Systematic Biology 54 (2), 241–253.

Miller, R.R., 1959. Origin and affinities of the freshwater fish fauna of western North America. American Association for the Advancement of Science Publication 51, 182–222.

Miller, R.R., Smith, M.L., 1986. Origin and zoogeography of the fishes of central Mexico. In: Hocutt, C.H., Wiley, E.O. (Eds.), The Zoogeography of North American Freshwater Fishes. John Wiley and Sons, New York, pp. 487–517.

Miller, R.R., Minckley, W.L., Norris, S.M., 2006. Freshwater Fishes of Mexico, first ed. University of Chicago Press, Chicaog, IL, pp. 490.

Nelson, E.M., 1948. The comparative morphology of the Weberian apparatus of the Catostomidae and its significance in systematics. Journal of Morphology 83, 225–251.

Nelson, E.M., 1949. The opercular series of the Catostomidae. Journal of Morphology 85, 559–567.

Oakley, T.H., Phillips, R.B., 1999. Phylogeny of salmonine fishes based on growth hormone introns: Atlantic (*Salmo*) and Pacific (*Oncorhynchus*) salmon are not sister taxa. Molecular Phylogenetics and Evolution 11, 381–393.

Robins, R.R., Raney, E.C., 1956. Studies of the catostomid fishes of the genus *Moxostoma*, with descriptions of two new species. Cornell University Agricultural Experiment Station, Memoir 34327, 1–56.

Robins, R.R., Raney, E.C., 1957. The systematic status of the suckers of the genus *Moxostoma* from Texas, New Mexico, and Mexico. Tulane Studies in Zoology 5 (12), 291–318.

Shimodaira, H., 2008. Testing regions with nonsmooth boundaries via multiscale bootstrap. Journal of Statistical Planning and Inference 138 (5), 1227–1241.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Molecular Biology and Evolution 16, 1114–1116.

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, pp. 778–813.

Smith, M.L., Miller, R.R., 1986. The evolution of the Rio Grande basin as inferred from its fish fauna. In: Hocutt, C.H., Wiley, E.O. (Eds.), The Zoogeography of North American Freshwater Fishes. John Wiley and Sons, New York, pp. 457–485.

Stamatakas, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22 (21), 2688–2690.

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

Walsh, P.S., Metzger, D.A., Higuchi, R., 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10 (4), 506–513.