



Multilocus molecular phylogeny of the suckermouth armored catfishes (Siluriformes: Loricariidae) with a focus on subfamily Hypostominae



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ABSTRACT

The Neotropical catfish family Loricariidae is the fifth most species-rich vertebrate family on Earth, with over 800 valid species. The Hypostominae is its most species-rich, geographically widespread, and ecologically diverse subfamily. Here, we provide a comprehensive molecular phylogenetic reappraisal of genus-level relationships in the Hypostominae based on our sequencing and analysis of two mitochondrial and three nuclear loci (4293 bp total). Our most striking large-scale systematic discovery was that the tribe Hypostomini, which has traditionally been recognized as sister to tribe Ancistrini based on morphological data, was nested within Ancistrini. This required recognition of seven additional tribe-level clades: the *Chaetostoma* Clade, the *Pseudancistrus* Clade, the *Lithoxus* Clade, the '*Pseudancistrus*' Clade, the *Acanthicus* Clade, the *Hemiancistrus* Clade, and the *Peckoltia* Clade. Results of our analysis, which included type- and non-type species for every valid genus in Hypostominae, support the reevaluation and restriction of several historically problematic genera, including *Baryancistrus*, *Cordylancistrus*, *Hemiancistrus*, and *Peckoltia*. Much of the deep lineage diversity in Hypostominae is restricted to Guiana Shield and northern Andean drainages, with three tribe-level clades still largely restricted to the Guiana Shield. Of the six geographically widespread clades, a paraphyletic assemblage of three contain lineages restricted to drainages west of the Andes Mountains, suggesting that early diversification of the Hypostominae predated the late Miocene surge in Andean uplift. Our results also highlight examples of trophic ecological diversification and convergence in the Loricariidae, including support for three independent origins of highly similar and globally unique morphological specializations for eating wood.

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1. Introduction

1.1. Overview

The Neotropical family Loricariidae is the most species-rich family of catfishes, containing over 800 valid species (Eschmeyer, 2014) and likely several hundred undescribed species. Loricariids are easily distinguished from other fishes by having bodies covered in ossified dermal plates, an abundance of integumentary teeth known as odontodes (Garg et al., 2010), and a ventral oral disk that facilitates surface attachment and feeding (Geerinckx et al., 2011). A host of morphological and molecular studies support the monophyly of Loricariidae and its placement among five other families

in the Neotropical endemic suborder Loricarioidei (Fig. 1A, Supplemental Table 1). Across these families, a sequential loss of three cranial biomechanical linkages has been correlated with an increase in morphological diversity (Schaefer and Lauder, 1996), which has made the Loricarioidei a prominent example of the positive effect that modularization can have on evolutionary diversification – first hypothesized by Darwin (1859 190–191). In addition to their potential role in promoting morphological diversification, the losses of biomechanical linkages from loricariid crania have contributed to a globally unique, highly specialized oral jaw that consists of bilaterally independent lower jaw rami and an independently operable upper jaw (Schaefer and Lauder, 1986; Adriaens et al., 2009; Lujan and Armbruster, 2012).

Loricariids are typically small- to medium-sized fishes (<20 cm SL), although some species reach almost 1 m in total length (Lujan et al., 2010). Many species are boldly patterned or distinctively shaped and are exported in large numbers to the international

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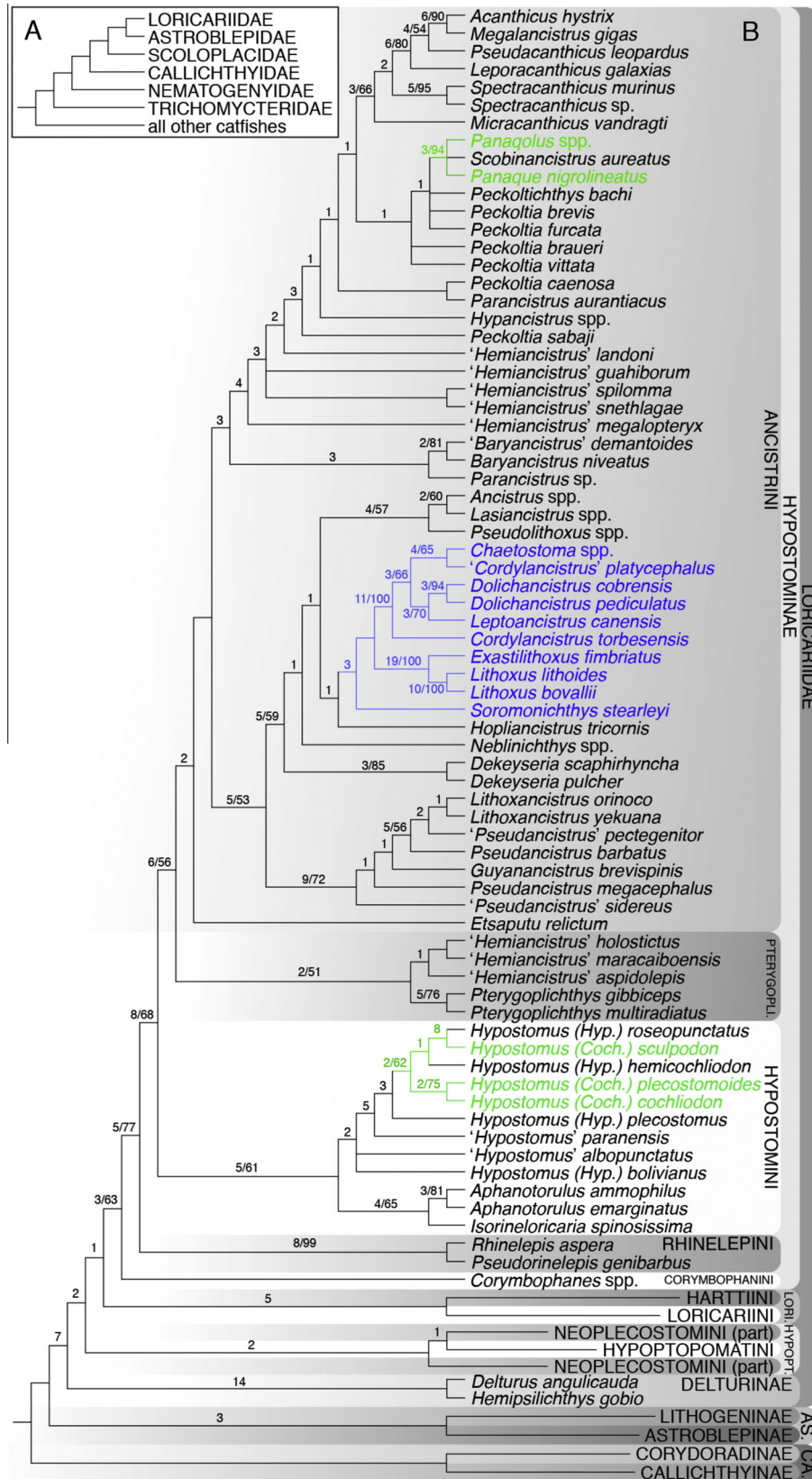


Fig. 1. (A) Family-level phylogenetic relationships of the Neotropical catfish suborder Loricarioidei following Sullivan et al. (2006). (B) A hybrid of the hypothesized phylogenetic relationships within the family Loricariidae proposed by Armbruster (2004a, 2008). Numbers are decay indices followed by bootstrap values (if bootstrap values are >50%). 'Trans-Highland Clade' in blue, and specialized wood-eating lineages in green (see text for further explanation). Abbreviations: AS. = Astroblepidae, CA. = Callichthyidae, Coch. = *Cochliodon*, Hyp. = *Hypostomus*, LORI. = Loricariinae, HYPOPT. = Hypoptopomatinae, PTERYGOPLI. = Pterygoplichthyini. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Primers and annealing temperatures (T_a) used to amplify and sequence each of the loci in this study.

Locus	Primer name	Primer sequence (5'→3')	T_a (°C)	Reference	Coverage	Specificity	Notes
16S	16Sa	CGCCTGTTTATCAAAAACAT	50	Palumbi et al. (2002)	Partial (5' end)	None	
	16Sb	CCGGTCTGAACCTCAGATCACGT		Palumbi et al. (2002)	Partial (5' end)	None	
Cyt <i>b</i>	CytbFa	TCCCACCCGGACTCTAACCGA	57	This study	Entire	Loricarioidei	First choice
	CytbFb	CCCACCCGGACTCTAACCGA	57	This study	Entire	Loricarioidei	Second choice
	CytbFc	CGCCCTAATTGATCTCCCG	57	This study	Entire	Loricarioidei	
	CytbRa	CCGGATTACAAGACCCGGCT	57	This study	Entire	Loricarioidei	
	CytbRc	CTCCGGATTACAAGACCCGGC	57	This study	Entire	Loricarioidei	
MyH6	myh6_F459	CATMTTYCCATCTCAGATAATGC	53	Li et al. (2007)		None	First PCR forward primer
	myh6_F507	GGAGAATCARTCKGTGCTCATCA	62	Li et al. (2007)		None	Second PCR forward primer
	myh6_R1325	ATTCTCACCACTCCAGTTGAA	53	Li et al. (2007)		None	First PCR reverse primer
	myh6_R1322	CTCACCACTCCAGTTGAACAT		Li et al. (2007)		None	Second PCR reverse primer
RAG1	RAG1Fa	CCTGGTTTTATGCATTTGAGTGCCA	53	This study	Exon 3	Loricariidae	
	RAG1R186	ACGCTCTTCTGARGGAACTA		This study	Exon 3	Loricariidae	
	RAG1Ra	AGGGCATCTAATGTGGGCTGTGT		This study	Exon 3	Loricarioidei	
RAG2	RAG2Fc	ATGGAGGCCGAACACCAACA	58	This study	Entire	Loricarioidei	
	RAG2R961	CGTGCTGWACTCCATTT		This study	Entire	Loricarioidei	

ornamental fish trade (Prang, 2007), where individual fish regularly fetch hundreds to thousands of dollars (NKL, pers. obs). Loricariids are ubiquitous inhabitants of lotic habitats throughout the Atlantic-slope of South America north of Buenos Aires, the Pacific-slope of South America north of Peru, and Central American drainages south of Costa Rica. They are obligate bottom-feeders and consume an often indistinguishable mix of detritus and algae (Buck and Sazima, 1995). However, diverse sympatric assemblages may partition benthic food resources along cryptic axes that are only chemically discernable (Lujan et al., 2012), and several lineages show morphological specializations for the consumption of specific foods including wood, seeds, and macroinvertebrates (Lujan et al., 2011). With their extensive exoskeletal armor and dense endoskeleton, loricariid bodies have the highest calcium phosphate concentrations of any measured fishes (Vanni et al., 2002), which make them an ecologically important regulator of primary production and nutrient dynamics in many Neotropical rivers (Knoll et al., 2009). Despite their incredible diversity, intriguing morphology, and ecological significance, the evolutionary origins of the Loricariidae remain poorly understood. A robust and well-resolved phylogenetic hypothesis is needed to facilitate systematic and evolutionary research into both this distinctive group of fishes and the Neotropical ecosystems of which they are an integral part.

1.2. Systematics

Since the first major monograph on loricariid systematics by Regan (1904), there have been at least 36 phylogenetic studies of relationships within the Loricariidae, and these have been divided approximately equally between studies based on morphological vs. molecular data (Supplemental Table 1). Both data sets provide generally consistent support for placement of the Loricariidae among five other families in the siluriform suborder Loricarioidei (Fig. 1A, de Pinna, 1998; Sullivan et al., 2006), and for division of the Loricariidae into six clades typically assigned the rank of subfamily (Fig. 1B): Lithogeninae, Delturinae, Hypoptopomatinae, Neoplecostominae, Loricariinae, and Hypostominae. However, disagreements persist about the composition of even these higher-level clades (e.g., placement of Lithogeninae in Astroblepidae or Loricariidae; Schaefer, 2003 vs. Armbruster, 2004a and Hardman, 2005). Morphological–molecular discordance and homoplasy is much more problematic at finer taxonomic scales (e.g., genus *Pseudancistrus* comprising a single genus, Armbruster (2008), or five genera, Covain and Fisch-Muller (2012)). Synapomorphies for

many clades below the rank of tribe are scarce (see decay indices in Fig. 1B, Armbruster, 2004a, 2008) and there is ongoing disagreement about the monophyly and taxonomic validity of many genera.

Regardless of this systematic confusion, taxonomists continue to discover and describe many new genera (e.g., Rodriguez et al., 2011; Ribeiro et al., 2012; Salcedo, 2013). Indeed, unflagging rates of taxonomic discovery have exacerbated systematic confusion and the difficulty of obtaining broadly representative specimens for large-scale comparative research. Taxonomic revisions and phylogenetic analyses have therefore typically focused on one or a few of the consistently recognized subfamilies (e.g., Loricariinae: Covain, 2011; Rodrigues et al., 2011; Hypoptopomatinae: Chiachio et al., 2008; Cramer et al., 2011). Comprehensive phylogenetic examinations of the Hypostominae – the most species rich, geographically widespread, and ecomorphologically diverse subfamily – have thus far been limited to the morphology-based studies of Armbruster (2004a, 2008; Armbruster and Taphorn, 2011; Fig. 1B).

One challenge to comprehensive review of the Hypostominae has been this clade's great and growing diversity, currently spanning at least 40 valid genera (this study) and over 400 valid species (Eschmeyer, 2014). Recent Hypostominae systematics have been dynamic and sometimes contradictory: Armbruster (2004a) synonymized over a dozen Hypostominae genera, some of which had only recently been described, and recognized as valid several genera that were themselves either poorly supported or paraphyletic (e.g., *Cordylancistrus*, *Hemiancistrus*, *Baryancistrus*, *Peckoltia*; Fig. 1B). To date, only 27 of the 36 Hypostominae genera recognized as valid in this study have been examined in one or another of nine previous molecular phylogenetic studies (Supplemental Table 1). Of those studies, six have examined only mitochondrial DNA loci (e.g., ribosomal 12S and 16S, Cytochrome *b* [Cyt *b*], control region [D-loop], NADH Dehydrogenase subunit 4 [ND4], and tRNAs), and only three have also examined nuclear markers (e.g., Recombination Activation Gene subunits 1 and 2 [RAG1, RAG2], Reticulon-4 [RTN4]).

1.3. Macroevolutionary hypotheses

In addition to systematic insights, a novel DNA-based model of evolutionary relationships provides an opportunity to reexamine a biogeographical and an ecomorphological hypothesis that are currently only supported by morphology-based taxonomic and phylogenetic evidence. The first – the 'Trans-Highland Clade' hypothesis (originally proposed by Lujan and Armbruster, 2011a,b) – states

that a monophyletic group of mostly Andean-distributed genera (the *Chaetostoma* group: *Chaetostoma*, *Cordylancistrus*, *Dolichancistrus*, *Leptoancistrus*) had its origin in the remote and more geologically ancient highlands of the Guiana Shield. This hypothesis is supported exclusively by biogeographical patterns apparent in the phylogeny of Armbruster (2008; Fig. 1B). Armbruster (2008) found the *Chaetostoma* group to be sister to a clade entirely restricted to the Guiana Shield (*Lithoxus* + *Exastilithoxus*), with the next sister lineage (*Soromonichthys stearleyi*) also being restricted to the Guiana Shield (Fig. 1B).

The second hypothesis – the ‘Diphyletic Wood-Eaters’ hypothesis – is also a direct extension of Armbruster’s (2004a, 2008) phylogenies, which found that morphological specializations for wood-eating (e.g., adze-shaped teeth, force-maximizing jaw geometries, and diets consisting largely of wood particles; Lujan et al., 2011; Lujan and Armbruster, 2012) arose only twice in Loricariidae: Once in the tribe Hypostomini, at the base of a putatively monophyletic group within *Hypostomus* (Fig. 1B; i.e., the *Hypostomus cochliodon* group), and once in the tribe Ancistrini, at the base of a putatively monophyletic group containing *Panaque*, *Panaqolus*, and *Scobinancistrus* (Fig. 1B). For either of these hypotheses, a phylogenetic topology that substantially differs from those of Armbruster (2004a, 2008) would likely either weaken or remove support.

1.4. Goals

Our goals are to (1) perform a comprehensive molecular reappraisal of relationships and monophyly of subfamilies and tribes throughout the Loricariidae by including representatives of every previously recognized subfamily and tribe in Loricariidae as ingroups and representatives of three other loricarioid families as outgroups (including a total of 181 species of Loricariidae representing 84 currently valid genera); (2) reexamine tribe- and genus-level monophyly and relationships within the Hypostominae (including 145 species and 45 nominal genera in Hypostominae), and (3) test morphology-based phylogenetic topologies that support the two macroevolutionary hypotheses described above. Of the lineages examined in this study, many have never before been examined phylogenetically, and 13 currently valid genera have never previously been examined using molecular methods, making this the most taxonomically comprehensive phylogenetic analysis of the Loricariidae to date.

2. Methods

2.1. Taxon sampling

Our objective in taxonomic sampling was to comprehensively sample genera within Hypostominae and to maximize the breadth of genera representing other loricariid subfamilies and tribes. Given current confusion surrounding the validity and boundaries of many Hypostominae genera, we attempted to maximize the number of type species included for each genus and the number of lineages previously recognized within paraphyletic genera. This included representatives of most genera found to be junior synonyms by Armbruster (2004a). Summaries of taxa included in this study are complicated by highly variable taxonomic interpretations across previous studies and current sources (e.g., Eschmeyer, 2014). We therefore provide our own comprehensive summary of genera, tribes, and subfamilies according to their inclusion in this and each of 38 previous phylogenetic studies of the Loricariidae (Supplemental Table 1).

Sequence data for many taxa outside the Hypostominae, including most members of the Rhinelepineae, Loricariinae, Hypop-

topomatinae, and Lithogeninae were obtained from previously published studies (e.g., Hardman, 2005; Sullivan et al., 2006; Cramer et al., 2011) via the Genbank sequence database. Newly generated sequence data were obtained from tissue samples or DNA extracts collected by the authors or provided by the Academy of Natural Sciences of Drexel University in Philadelphia, PA (ANSP), the Museu de Ciências e Tecnologia at the Pontifícia Universidade Católica do Rio Grande do Sul in Porto Alegre, Brazil (MCP), the Muséum d’Histoire Naturelle in Geneva, Switzerland (MHNG), and the Smithsonian Tropical Research Institute in Panama (STRI), or obtained through the ornamental fish trade. Voucher specimens for each tissue were identified either directly by the first author, directly by curators and collection managers at contributing institutions, or by exchange of photographs. Whenever possible, multiple individuals of the same species were included in initial analyses for quality control, but most supernumerary individuals were subsequently removed if sequence data were invariant (see Section 2.4 below). Species identifications of Genbank data were not reevaluated, although names were updated to reflect current taxonomy.

2.2. Marker selection

Two genes were selected from the mitochondrial genome (16S, *Cyt b*), and three from the nuclear genome (RAG1, RAG2, MyH6) based on their use in previous phylogenetic studies – to maximize availability of primers and comparative data – and on their representation of a wide range of evolutionary mutation rates – to maximize resolution at all depths of the phylogeny. We amplified and sequenced an approximately 600 bp fragment of the 16S gene located near the 5’ end of the gene. We also amplified and sequenced an approximately 1150 bp fragment of the mtDNA genome spanning the entire *Cyt b* gene and neighboring portions of the glutamyl-tRNA at the 3’ end and threonine-tRNA at the 5’ end. Additionally, we amplified an approximately 1020 bp fragment of the nuclear gene RAG1 encompassing most of exon 3 (Sullivan et al., 2006), an approximately 950 bp fragment encompassing the entire RAG2 gene (Sullivan et al., 2006), and an approximately 660 bp fragment encompassing a portion of the MyH6 gene (Li et al., 2007). Each fragment was amplified using combinations of previously published and newly developed primers (Table 1).

2.3. DNA extraction, amplification and sequencing

Whole genomic DNA (gDNA) was extracted from fin or muscle tissues preserved in 95% ethanol following manufacturer’s instructions for the DNeasy Blood & Tissue Kit (Qiagen N.V., Venlo, Netherlands). Fragment amplifications were performed in either 12.5 or 25 µl reactions containing 75.4% (by volume) dH₂O, 10% Erika Hagelberg (EH) buffer (Hagelberg, 1994; 10 mM), 4% forward primer (10 mM), 4% reverse primer (10 mM), 2.2% deoxyribonucleotide triphosphate (dNTP) mix (10 mM; New England Biolabs, Ipswich, MA), 0.4% *Taq* polymerase (LTI: Life Technologies Inc., Carlsbad, CA), and 4% extracted gDNA template. 16S, *Cyt b*, RAG1, and RAG2 genes were amplified via standard polymerase chain reaction (PCR) using an Eppendorf Mastercycler[®] pro S thermocycler (Eppendorf Ltd., Hamburg, Germany) with an initial denaturation step of 3 min at 94 °C, followed by 35 cycles of 94 °C for 30 s, annealing at various temperatures (see Table 1) for 30 s, and extension at 72 °C for 45 s, followed by a final extension step at 72 °C for 5 min. MyH6 was amplified via two-stage nested PCR with the product of the first PCR being diluted 100× before use as template in the second PCR. For all genes, the entire volume of PCR product was run on a 1% agarose gel with 0.01% SYBR[®] Safe DNA gel stain (LTI). The band corresponding to the target locus was cut from the gel and the target PCR product extracted by

centrifuge filtration through a trimmed P-200 pipet filter tip in a 1 ml snap-top tube (5 min at 15,000 rpm; Dean and Greenwald, 1995).

Forward and reverse sequencing reactions were conducted separately in 10 µl reactions containing 2 µl dH₂O, 2 µl 5× sequencing buffer (LTI), 0.5 µl primer (10 mM), 0.5 µl BigDye[®] terminator (LTI), and 5 µl PCR product. All sequencing reactions followed the manufacturer's recommended thermocycler profile (initial denaturation step of 1 min at 96 °C, followed by 30 cycles of 96 °C for 10 s, annealing at 50 °C for 5 s, and extension at 60 °C for 4 min). Precipitation of the sequencing product also followed manufacturer's recommendations. Briefly, 2 µl of a 50/50 mixture of 3 M sodium acetate (pH 5.2) and 125 mM EDTA (pH 8.0) plus 25 µl of 96% ethanol were added to the sequencing product. The resulting mixture was transferred to a MicroAmp[®] optical 96-well reaction plate (LTI) and centrifuged for 30 min at 4000 rpm. The supernatant was decanted and the plate spun upside down for 1 min at 100 g to remove excess supernatant. 35 µl of 70% ethanol was added to each well and the plate again centrifuged for 15 min at 4000 rpm, the supernatant again decanted, and the plate spun upside down for 1 min at 100 g. All centrifugation steps were performed at 4 °C. A final incubation step for 1 min at 95 °C was performed to evaporate residual ethanol. 10 µl of Hi-Di[™] formamide (LTI) was added and pipette-mixed and the plate incubated for 2 min at 95 °C before being loaded into an Applied Biosystems[™] 3730 DNA analyzer (LTI) for sequencing.

2.4. Sequence assembly, alignment, and phylogenetic inference

Contigs were automatically assembled from chromatograms for forward and reverse sequences using Geneious[®] software (v6.1.7, Biomatters Ltd., Auckland, New Zealand). Following automatic assembly, each contig was checked visually. Sequences that did not overlap or that had unusually high numbers of ambiguities were reamplified and resequenced. Consensus sequences were then extracted and compared with sequences from other individuals of the same species whenever possible. If contigs for a given sequence or individual did not closely agree with other individuals, the sequence was either excluded or reamplified and resequenced. Sequence quality was confirmed by the analysis of neighbor-joining networks generated from all-individual, all-taxon alignments of individual loci. Species that were reciprocally monophyletic for all loci were reduced to a single individual in the final analysis.

Contigs for all sequences were first aligned using the MUSCLE algorithm (Edgar, 2004) as implemented in Geneious[®] and alignments were then manually edited. Manual editing of the 16S alignment was done according to the secondary structural model of *Xenopus laevis* following López-Fernández et al. (2005). Only unambiguously alignable regions were included; hypervariable, unalignable loop regions (totaling approximately 60 bp) were excluded. Alignments of protein coding gene sequences were evaluated based on their amino acid translation with gaps being aligned to codons. Only aligned sequences with open reading frames were included in analyses. Alignments of all loci were concatenated to create a single alignment consisting of 4293 bp and 215 individuals (181 species, 91 genera). In cases where a sequence was available for one individual and not another of the same species, sequences from different individuals of the same species were concatenated. A complete list of individuals and loci sequenced and combined for each individual are provided in Table 3. The final alignment had 82% gene by individual data coverage (# of individuals/# of sequences), with 93% gene by individual coverage across individuals sequenced *de novo* for this study, and 42% gene by individual coverage across individuals whose data were downloaded from Genbank.

PartitionFinder (v1.1.1, Lanfear et al., 2012) was used to determine codon-specific models of molecular evolution for each gene under the Bayesian information criterion (BIC). A generalized time reversible model with a proportion of invariable sites and rate heterogeneity of the remainder being modeled by a gamma distribution (GTR + I + Gamma) was determined to be the best model of molecular evolution for 16S (all sites), Cyt *b* (all sites), the first two codon positions of RAG1 and RAG2, and the first and third codon positions of MyH6. A GTR model with rate heterogeneity of all sites being modeled by a gamma distribution (GTR + Gamma) was determined to be the best model of molecular evolution for the third codon positions of RAG1 and RAG2 and the second codon position of MyH6. All data partitions were unlinked with rates free to vary across partitions.

Phylogenetic analysis of the concatenated alignment was conducted using both Bayesian inference (BI) and maximum likelihood (ML) methods with *Vandellia* sp. (Trichomycteridae) designated as the outgroup. A Bayesian Markov chain Monte Carlo search of tree space was conducted using MrBayes (v3.2.2; Ronquist and Huelsenbeck, 2003) on the CIPRES supercomputing cluster (Miller et al., 2010). MrBayes was programmed to run for 50 million generations using eight chains (nchain = 8; i.e., two parallel runs with 1 cold and 7 hot chains each; temperature parameter set to default), sampling every 3500 trees with the first 30% of trees (4285) being discarded as burnin. The Bayesian search was determined to have reached stationarity when cold chains stopped increasing and randomly fluctuated within a stable $-\ln$ range of values and when effective sample size for all metrics exceeded 200 as determined in the software Tracer (v1.6; Rambaut et al., 2007). Maximum likelihood analysis was conducted using RAxML (v8.0.0; Stamatakis, 2014) programmed to first conduct a 200 generation search for the best tree and then generate bootstrap support values based on a 2000 generation search of tree space.

2.5. Taxonomic decisions

Our taxonomic decisions were guided by a desire to maximize congruence between priority as determined by the International Code of Zoological Nomenclature and phylogenetic relationships inferred from molecular genetic data. Priority, statistical support for a given node, and congruence between independent phylogenetic analyses were considered. We refrained from describing new genera or diagnosing clades using molecular characters as an analysis combining our results with previously documented morphological traits will be provided elsewhere. Several taxa examined in this study are undescribed genera or species that have previously been recognized as distinct by aquarium fish hobbyists and been assigned a standardized alphanumeric code (an L-number; Dignall, 2014) as a way of tracking them pending official description. Given the utility and generally standardized application of these codes we have adopted them throughout this manuscript.

3. Results

3.1. Reappraisal of Loricariidae subfamilies and tribes, Fig. 2 and Table 2

Both BI and ML analyses supported a monophyletic Loricariidae inclusive of Lithogeninae (Node 201: BI: 0.96, ML: 78), and sister relationship between Loricariidae and Astroblepidae (Node 206: BI: 1.0, ML: 98). The exact location of type species *Lithogenes villosus* within Loricariidae was unresolved, with both BI and ML finding it in a three-way polytomy with Delturinae and all other Loricariidae (Node 201). Both analyses supported major clades

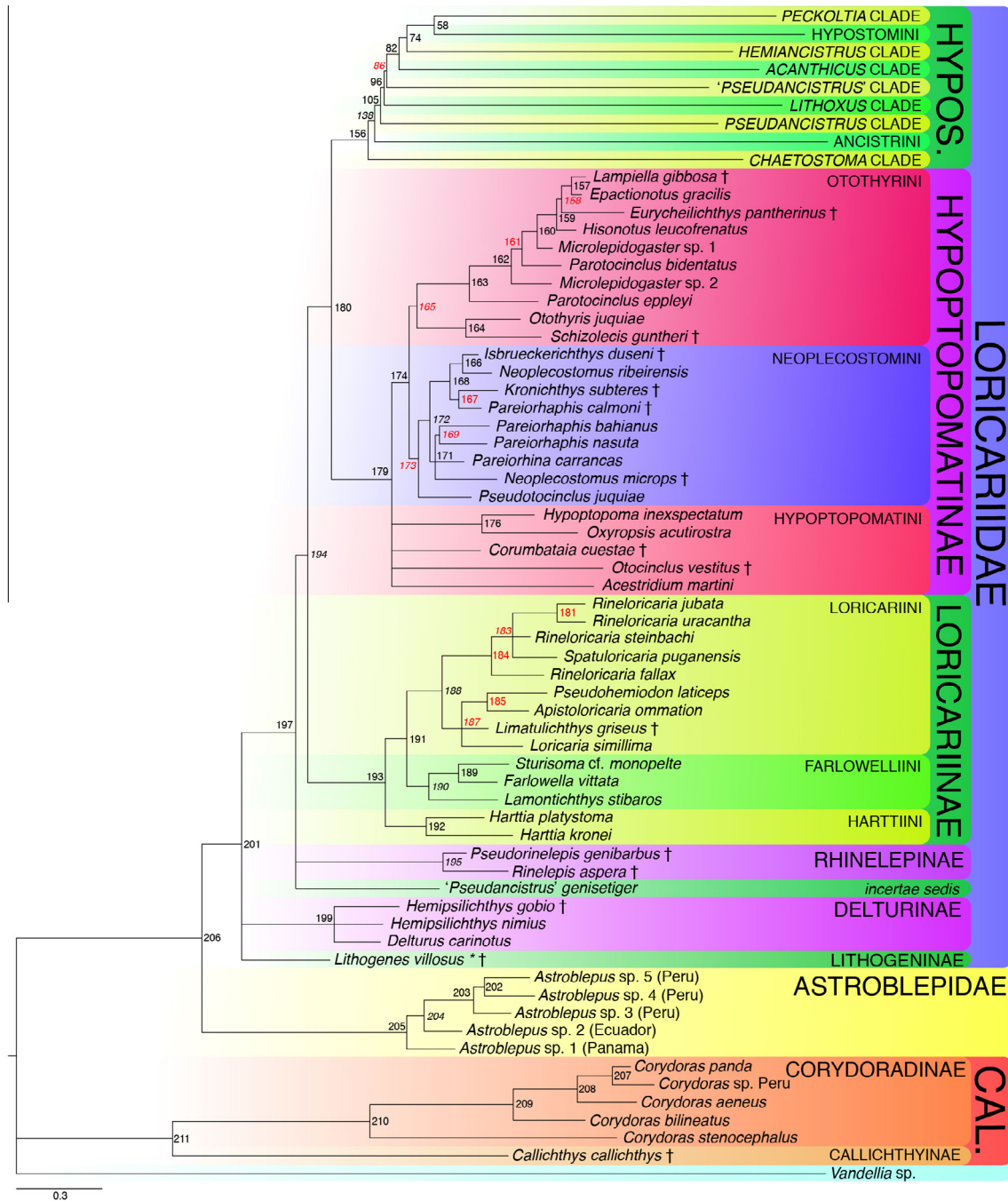


Fig. 2. Phylogenetic relationships of the Neotropical catfish family Loricariidae based on Bayesian analysis of a 4293 base pair alignment consisting of two mitochondrial (16S, Cyt *b*) and three nuclear loci (RAG1, RAG2, MyH6). Node numbers correspond to Bayesian posterior probability (BI) and maximum likelihood (ML) support values in Table 2. Numbers in red indicate BI < 0.90; numbers in italics indicate ML < 50. Samples taken from at or near the type locality for a given species are indicated by asterisks (†) and species that are types for their genus are indicated by crosses (†). Relationships among the Hypostominae are expanded in Figs. 3 and 4, with background color corresponding to each clade remaining unchanged. Abbreviations: CAL. = Callichthyidae, HYPOS. = Hypostominae.

within Loricariidae that were generally congruent with previous morphology-based delimitations of the subfamilies Delturinae (Node 199: BI: 1.0, ML: 75), Loricariinae (Node 193: BI: 1.0, ML: 98), Hypoptopomatinae (Node 179: BI: 1.0, ML: 97), and Hypostominae (Node 156: BI: 0.96, ML: 72); however, important differences in the arrangement and composition of these subfamilies remained. The putatively hypostomin genera *Rhinelepis* and *Pseudorinelepis* were excluded from the clade consisting of ((Hypostominae + Hypoptopomatinae) Loricariinae). The BI analysis found *Rhinelepis* and *Pseudorinelepis* to be a moderately supported clade (Rhinelepininae, Node 195: BI: 0.92) forming an

unresolved polytomy with '*Pseudancistrus*' *genisetiger* and a moderately supported clade consisting of all other Loricariidae exclusive of Delturinae and Lithogeninae. The ML analysis did not resolve relationships between *Rhinelepis* and *Pseudorinelepis* or between these genera and other Loricariidae (Supplemental Fig. 1). Given the conflicting support for placement of '*Pseudancistrus*' *genisetiger*, and the absence of specific morphological data, we treat this species as *incertae sedis* within Loricariidae.

Both BI and ML analyses found strong support for a monophyletic Loricariinae (Node 193: BI: 1.0, ML: 98), and found Loricariinae to be sister to Hypostominae + Hypoptopomatinae, although

ML support for the latter was poor (Node 194: BI: 0.96, ML: 43). Within Loricariinae, the tribe Harttiini *sensu* Rapp Py-Daniel (1997) was found to be paraphyletic and Loricariini *sensu* Rapp Py-Daniel (1997) and Covain (2011) was ambiguously supported as monophyletic (Node 188: BI: 1.00, ML: 32) and sister to Farlowelliini (Node 191: BI: 1.00, ML: 72).

Both BI and ML analyses found strong support for a monophyletic Hypoptopomatinae inclusive of Neoplecostominae (Node 179: BI: 1.00, ML: 97), and found moderate support for a sister relationship between Hypoptopomatinae *sensu lato* and Hypostominae (Node 180: BI: 0.95, ML: 58). Monophyly of Neoplecostominae *sensu* Armbruster (2004a; found to be paraphyletic therein) was moderately supported (Node 173: BI: 0.85, ML: 28) and this clade was sister to Otothyriini *sensu* Schaefer (1998; Node 174: BI: 1.00, ML: 86). The clade Otothyriini + Neoplecostomini was found to be sister to a paraphyletic assemblage of other genera traditionally included in tribe Hypoptopomatini *sensu* Schaefer (1998; Node 179; e.g., *Acestridium*, *Corumbataia*, *Hypoptopoma*, *Oxyropsis*). Within this assemblage, strong support was only found for a sister relationship between *Hypoptopoma* and *Oxyropsis* (Node 176: BI: 1.00, ML: 100), which is consistent with morphology-based relationships hypothesized by Schaefer (1998).

Hypostominae *sensu stricto* (i.e., exclusive of Rhinelepininae and 'Pseudancistrus' *genisetiger*) was moderately supported as monophyletic (Node 156: BI: 0.96, ML: 72). Within Hypostominae, relationships among genera significantly differ from previous morphology-based hypotheses. Both BI and ML analyses clustered genera into nine congruent and generally well-supported clades that we discuss sequentially.

3.2. Reappraisal of Hypostominae tribes and genera

3.2.1. The *Chaetostoma* Clade, Fig. 3 and Table 2

The *Chaetostoma* group *sensu* Armbruster (2004a, 2008) was weakly supported as monophyletic (Node 155: BI: 0.66, ML: 64) and sister to all other Hypostominae, albeit with moderate ML support (Node 156: BI: 0.96, ML: 72). This contrasts with the strong support for monophyly derived from morphological data (e.g., Armbruster, 2004a, 2008; Fig. 1B; decay index = 11, bootstrap support = 100). Generic composition of the *Chaetostoma* group remains the same as in Armbruster (2004a, 2008; i.e., *Chaetostoma*, *Cordylancistrus*, *Dolichancistrus*, *Leptoancistrus*), plus the recently described genus *Loraxichthys*. The validity of *Loraxichthys* plus *Lipopterychthys* (the latter synonymized with *Chaetostoma* by Armbruster, 2004a, and resurrected by Salcedo, 2013), were reexamined. The morphology-based phylogenetic analysis by Salcedo (2013) erected the new genus *Loraxichthys* and found it and *Lipopterychthys* to form a clade sister to *Leptoancistrus*. We found *Lipopterychthys* and *Loraxichthys* to be independently nested within a strongly-monophyletic genus *Chaetostoma* (Node 149: BI: 1.0, ML: 100). *Chaetostoma* itself was divided into two reciprocally monophyletic and geographically distinctive clades: a well-supported clade of northern Pacific Coast (*Ch. n.sp.* Guayas), Panamanian (*Ch. fischeri*), and Guiana Shield (*Ch. vasquezi*) species (Node 148: BI: 0.99, ML: 59), and a moderately supported clade of species distributed across the Atlantic slope of the Andes Mountains from Colombia to southern Peru (Node 145: BI: 0.82, ML: 56).

Chaetostoma was sister to a clade containing the genera *Cordylancistrus*, *Dolichancistrus*, and *Leptoancistrus* (Node 154: BI: 0.87, ML: 72). *Cordylancistrus* was found to be paraphyletic in both the most recent morphological analyses (Armbruster, 2008; Salcedo, 2013) and in this study: The type species *Cordylancistrus torbesensis* was well-supported as sister to *Dolichancistrus* (Node 151: BI: 0.99, ML: 88). *Leptoancistrus* was strongly supported as sister to *Co. torbesensis* + *Dolichancistrus* (Node 152: BI: 1.0, ML: 100), forming a northern Pacific-slope clade sister to the strongly monophyletic

clade of central trans-Andean (respectively Atlantic and Pacific slope) species: '*Cordylancistrus*' *platycephalus* + '*Co.*' *santarosensis* (Node 153: BI: 1.0, ML: 100).

3.2.2. Tribe Ancistrini Fig. 3 and Table 2

Tribe Ancistrini *sensu* Armbruster (2004a, 2008; Fig. 1B) was found to be paraphyletic, and is therefore restricted to a weakly supported clade (Node 137: BI: 0.75, ML: 11) containing the genera *Ancistrus*, *Corymbophanes*, *Dekeyseria*, *Guyanancistrus*, *Hopliancistrus*, *Lithoxancistrus*, *Lasiancistrus*, *Neblichthys*, *Paulasquama*, *Pseudolithoxus*, and *Soromonichthys*. Of these genera, multiple species or populations of the genera *Ancistrus*, *Lasiancistrus*, *Pseudolithoxus*, *Hopliancistrus*, *Guyanancistrus*, *Dekeyseria*, *Neblichthys* and *Lithoxancistrus* were examined and in all cases were well-supported as monophyletic (respective Nodes 112, 114, 122, 124, 127, 129, 133, 136; BI: >0.95, ML: >58). The monotypic genus *Soromonichthys* was nested within *Pseudolithoxus*, suggesting that the former genus may not be valid.

3.2.3. The *Pseudancistrus* Clade Fig. 3 and Table 2

The *Pseudancistrus* Clade, including the type species for *Pseudancistrus* (*Ps. barbatus*) and seven congeneric species or populations, was found to be strongly monophyletic (Node 104: BI: 1.0, ML: 100) and only distantly related to several genera that had been synonymized with *Pseudancistrus* (Armbruster, 2004a, 2008) but were recognized as valid by Covain and Fisch-Muller (2012): i.e., *Guyanancistrus* and *Lithoxancistrus*, in the newly redefined tribe Ancistrini; '*Pseudancistrus*' *pectegenitor* and '*Ps.*' *sidereus*, which are now recognized as an independent tribe-level clade; and '*Pseudancistrus*' *genisetiger*, which is now removed from Hypostominae.

3.2.4. The *Lithoxus* Clade Fig. 3 and Table 2

The *Lithoxus* Clade, containing the genera *Lithoxus* (represented by type species *L. lithoides* plus four putative congeners) and *Exastilithoxus* (represented by type species *E. fimbriatus* plus four putatively congeneric undescribed species or populations) was strongly supported as monophyletic (Node 95: BI: 1.0, ML: 98). *Exastilithoxus* was also well-supported as monophyletic (Node 90: BI: 1.0, ML: 100), with *L. lithoides* moderately supported as its sister (Node 91: BI: 0.90, ML: 50) and *L. jantjiae* weakly but consistently supported as sister to *L. lithoides* + *Exastilithoxus* (Node 92: BI: 0.51, ML: 28). Three eastern Guiana Shield species of *Lithoxus* (*L. cf. stocki*, *L. planquettei*, and *L. pallidimaculatus*) were strongly supported as monophyletic (Node 94: BI: 1.0, ML: 100) and sister to all other members of the *Lithoxus* Clade (Node 95: BI: 1.0, ML: 98).

3.2.5. The '*Pseudancistrus*' Clade Fig. 3 and Table 2

Although both '*Pseudancistrus*' *pectegenitor* and '*P.*' *sidereus* have ranges that overlap across much of the upper Orinoco and Casiquiare rivers, morphology-based phylogenetic evidence suggested that they were paraphyletic members of the *Pseudancistrus* clade *sensu* Armbruster (2008; Fig. 1B). Molecular data provide moderate support for monophyly of '*Pseudancistrus*' *pectegenitor* + '*P.*' *sidereus* (Node 85: BI: 1.0, ML: 73; Covain and Fisch-Muller, 2012), and relatively weak support for the position of this clade as sister to *Acanthicus*, *Hemiancistrus*, *Peckoltia*, and Hypostomini clades (Node 86: BI: 0.65, ML: 40). Nevertheless, molecular evidence supports the recognition of these species as a new genus.

3.2.6. The *Acanthicus* Clade Fig. 4 and Table 2

The *Acanthicus* Clade, containing the genera *Acanthicus*, *Leporacanthicus*, *Megalancistrus*, and *Pseudacanthicus*, was moderately to strongly supported as monophyletic (Node 81: BI: 0.99, ML: 83). Within this clade, monophyly of Amazon and Orinoco river genera (i.e., *Acanthicus*, *Leporacanthicus*, and *Pseudacanthicus*)

Table 3

Loci sequenced, voucher catalog number and country and river drainage of origin for the tissue samples analyzed in this study and summary of previously published data downloaded from GenBank. Boxes demarcate sequences concatenated from conspecific individuals.

Taxa	Tissue #	Topology	Type sp.	# of loci	16S	Cytb	RAG1	RAG2	Myb6	Voucher Cat #	Country	Drainage
Trichomycteridae												
<i>Vandellia</i> sp.	V5509			2	X	X			X	AUM 43867	Venezuela	Orinoco River
Callichthyidae												
Callichthyinae												
<i>Callichthys callichthys</i>	T10404			3	X	X		X		missing	Peru	Huallaga River
Corydoradinae												
<i>Corydoras aeneus</i>	T12836			4	X	X	X	X		ROM 90346	Bolivia	Mamoré River
<i>Corydoras bilineatus</i>	T12840			3	X	X	X	X		ROM 90344	Bolivia	Mamoré River
<i>Corydoras panda</i>	T12932			4	X	X	X	X		ROM 94924		
<i>Corydoras</i> sp. Peru	T10299			4	X	X	X	X	X		Peru	Madre de Dios River
<i>Corydoras stenocephalus</i>	T12839			5	X	X	X	X	X	ROM 90345	Bolivia	Mamoré River
Astroblepidae												
<i>Astroblepus</i> sp. 1	T9038			4	X	X	X		X	STRI 3803	Panama	Azucar River
<i>Astroblepus</i> sp. 2	T9028			4	X	X	X	X		STRI 3967	Ecuador	Zamora River
<i>Astroblepus</i> sp. 3	CH169			5	X	X	X	X	X	MUSM 44239	Peru	Huallaga River
<i>Astroblepus</i> sp. 4	CH161			5	X	X	X	X	X	missing	Peru	Huallaga River
<i>Astroblepus</i> sp. 5	CH173			5	X	X	X	X	X	MUSM 44237	Peru	Huallaga River
Loricariidae												
Lithoginiinae												
<i>Lithogenes villosus</i>	Genbank		†	1		X				Genbank	Guyana	Potaro River
Delturinae												
<i>Delturus carinotus</i>	Genbank			2			X	X		Genbank	Brazil	
<i>Hemipsilichthys gobio</i>	T14765			4	X	X	X	X	X	MCP 42452	Brazil	Pirapetinga River
<i>Hemipsilichthys nimius</i>	T14761			4	X	X	X	X	X	MCP 30671	Brazil	Perequê-Açú River
' <i>Pseudancistrus</i> '												
<i>'Pseudancistrus' genisetiger</i>	86.2			5	X	X	X	X	X	MHNG 2593.061	Brazil	São Francisco River
Rhinelepininae												
<i>Pseudorinelepis genibarbis</i>	Genbank		†	2	X	X				Genbank		
<i>Rhinelepis aspera</i>	Genbank		†	2			X	X		Genbank		
Loricariinae												
Harttiinae												
<i>Harttia kroniei</i>	Genbank			1			X			Genbank		
<i>Harttia platystoma</i>	T06287			4	X	X	X	X		ROM 85921	Guyana	Essequibo River
Farlowellinae												
<i>Farlowella vittata</i>	V5314			4	X	X	X	X		AUM 42218	Venezuela	Orinoco River
<i>Lamontichthys stibaros</i>	T10365			5	X	X	X	X	X	AUM 57480	Peru	Madre de Dios River
<i>Sturisoma</i> cf. <i>monopelte</i>	T06853			5	X	X	X	X	X	ROM 86207		
Loricariini												
<i>Apistoloricaria ommation</i>	Genbank			2			X	X		Genbank		
<i>Ixinandria steinbachi</i>	Genbank			2			X	X		Genbank		
<i>Limatulichthys griseus</i>	G5066		†	2	X				X	AUM 44405	Guyana	Essequibo River
<i>Loricaria similima</i>	Genbank			4	X	X	X	X		Genbank		
<i>Pseudohemiodon laticeps</i>	Genbank			1			X			Genbank		
<i>Rineloricaria fallax</i>	G5063			5	X	X	X	X	X	AUM 44444	Guyana	Essequibo River
<i>Rineloricaria jubata</i>	T13597			3	X	X		X	X	ROM 93680	Ecuador	Esmeraldas River
<i>Rineloricaria uracantha</i>	Genbank			2	X	X				Genbank		
<i>Spatuloricaria pугanensis</i>	Genbank			1		X				Genbank		
Hypoptopomatinae												
Hypoptopomatini												
<i>Acestridium martini</i>	Genbank			2			X	X		Genbank		
<i>Corumbataia cuestae</i>	Genbank		†	3	X	X	X			Genbank		
<i>Hypoptopoma inexpectatum</i>	Genbank			4	X	X	X	X		Genbank		
<i>Lampiella gibbosa</i>	Genbank			2			X	X		Genbank		
<i>Otocinclus vestitus</i>	Genbank		†	2			X	X		Genbank		
<i>Oxyropsis acutirostra</i>	Genbank			2			X	X		Genbank		
Neoplecostomini												
<i>Isbrueckerichthys duseni</i>	Genbank		†	4	X	X	X	X		Genbank		
<i>Kronichthys subteres</i>	Genbank		†	2	X		X			Genbank		
<i>Neoplecostomus microps</i>	Genbank		†	4	X	X	X	X		Genbank		
<i>Neoplecostomus ribeirensis</i>	Genbank			4	X	X	X	X		Genbank		
<i>Pareiorhaphis bahianus</i>	Genbank			2			X	X		Genbank		
<i>Pareiorhaphis calmoni</i>	Genbank		†	2			X	X		Genbank		
<i>Pareiorhaphis nasuta</i>	Genbank			2			X	X		Genbank		
<i>Pareiorhina carrancas</i>	Genbank			4	X	X	X	X		Genbank		
<i>Pseudotocinclus juquiaie</i>	Genbank			3	X	X		X		Genbank		
Otothyriini												
<i>Epactionotus gracilis</i>	Genbank			2			X	X		Genbank		
<i>Eurycheilichthys pantherinus</i>	Genbank			2			X	X		Genbank		
<i>Hisonotus leucofrenatus</i>	Genbank			3	X		X	X		Genbank		
<i>Lampiella gibbosa</i>	Genbank		†	2			X	X		Genbank		
<i>Microlepidogaster</i> sp. 1	Genbank			2			X	X		Genbank		
<i>Microlepidogaster</i> sp. 2	Genbank			2			X	X		Genbank		
<i>Otothyris juquiaie</i>	Genbank			2			X	X		Genbank		
<i>Parotocinclus bidentatus</i>	Genbank			2			X	X		Genbank		
<i>Parotocinclus eppleyi</i>	Genbank			1			X			Genbank		
<i>Schizolecis guntheri</i>	Genbank		†	2			X	X		Genbank		

(continued on next page)

Hypostominae									
Chaetostoma Clade									
<i>Cordylancistrus platycephalus</i>	T14019	*	5	X	X	X	ROM 93847	Ecuador	Santiago River
<i>Cordylancistrus santarosensis</i>	T13980	*	5	X	X	X	ROM 93798	Ecuador	Santa Rosa River
<i>Chaetostoma breve</i>	P6292		5	X	X	X	AUM 46515	Peru	Marañon River
<i>Chaetostoma cf. fischeri</i>	T9034		5	X	X	X	STRI 11581	Panama	Tuira River
<i>Chaetostoma dermorhynchum</i>	T14258		5	X	X	X	ROM 93656	Ecuador	Pastaza River
<i>Chaetostoma fischeri</i>	T9026	*	5	X	X	X	STRI 7604	Panama	Chagres River
<i>Chaetostoma lineopunctatum</i>	PE08047	*	5	X	X	X	MHNG 2712.041	Peru	Ucayali River
<i>Chaetostoma marmorescens</i>	CH198	*	5	X	X	X	MUSM 44898	Peru	Huallaga River
<i>Chaetostoma microps</i>	T14125	*	5	X	X	X	ROM 93895	Ecuador	Santiago River
<i>Chaetostoma n.sp. Guayas</i>	T13602	*	5	X	X	X	ROM 93687	Ecuador	Esmeraldas River
<i>Chaetostoma n.sp. Meta L445</i>	T12930		5	X	X	X	ROM 94925	Colombia	Meta River
<i>Chaetostoma vasquezii</i>	T09945	*	5	X	X	X	AUM 53812	Venezuela	Caura River
<i>Cordylancistrus torbesensis</i>	T674	* †	4	X	X	X	INHS 55478	Venezuela	Torbes River
<i>Dolichancistrus carnegiei</i>	6647		5	X	X	X	ANSP 189598	Colombia	Magdalena River
<i>Dolichancistrus fuesslii</i>	T14621	*	5	X	X	X	ROM 94484	Colombia	Guaviare River
<i>Leptancistrus canensis</i>	T9033	* †	5	X	X	X	STRI 11580	Panama	Tuira River
<i>Lipopterichthys carioni</i>	T14016	* †	5	X	X	X	ROM 93845	Ecuador	Santiago River
<i>Loraxichthys lexa</i>	PE08591	* †	5	X	X	X	MHNG 2712.071	Peru	Huallaga River
Ancistrini									
<i>Ancistrus bolivianus</i>	T12872		5	X	X	X	ROM 90368	Bolivia	Mamoré River
<i>Ancistrus clementinae</i>	T13829	*	5	X	X	X	ROM 93737	Ecuador	Guayas River
<i>Ancistrus leucostictus</i>	T08143		4	X	X	X	ROM 88561	Guyana	Essequibo River
<i>Ancistrus macrophthalms</i>	T09397	*	5	X	X	X	AUM 54994	Venezuela	Orinoco River
<i>Ancistrus megalostomus</i>	T10092		5	X	X	X	AUM 51165	Peru	Araza River
<i>Ancistrus ranunculus</i>	B1500	*	5	X	X	X	ANSP 199525	Brazil	Xingu River
<i>Ancistrus sp. Inambari</i>	T10383		5	X	X	X	AUM 57510	Peru	Inambari River
<i>Ancistrus sp. Xingu</i>	B1988		5	X	X	X	ANSP 199611	Brazil	Xingu River
<i>Corymbophanes kaiei</i>	T12637		5	X	X	X	ROM 89856	Guyana	Potaro River
<i>Dekeyseria pulchra</i>	V5296		5	X	X	X	AUM 44110	Venezuela	Atabapo River
<i>Dekeyseria scaphirhyncha</i>	T09540		5	X	X	X	AUM 54309	Venezuela	Ventuari River
<i>Dekeyseria scaphirhyncha</i>	T09861		4	X	X	X	AUM 54368	Venezuela	Orinoco River
<i>Guyanancistrus brevispinis</i>	86.1	* †	5	X	X	X	MHNG 2725.099	French Guiana	Maroni River
<i>Guyanancistrus longispinis</i>	85.7		5	X	X	X	MHNG 2725.100	French Guiana	Oyapock River
<i>Guyanancistrus niger</i>	85.6		5	X	X	X	MHNG 2722.089	French Guiana	Oyapock River
<i>Hoplancistrus n.sp. Xingu L017</i>	B2167		5	X	X	X	ANSP 193087	Brazil	Xingu River
<i>Hoplancistrus tricornis</i>	T9017	†	5	X	X	X	AUM 39853		aquarium specimen
<i>Lastancistrus guapore</i>	T14769		3	X	X	X	MCP 35652	Brazil	Purús River
<i>Lastancistrus schomburgkii</i>	P6125		5	X	X	X	AUM 45548	Peru	Marañon River
<i>Lastancistrus tentaculatus</i>	T09686		5	X	X	X	AUM 53895	Venezuela	Ventuari River
<i>Lithoxancistrus orinoco</i>	T09663	†	5	X	X	X	AUM 54439	Venezuela	Ventuari River
<i>Lithoxancistrus yekuana</i>	T9004	*	5	X	X	X	AUM 39473	Venezuela	Ventuari River
<i>Neblichthys breviracchium</i>	T06068	*	5	X	X	X	ROM 83692	Guyana	Mazaruni River
<i>Neblichthys echinatus</i>	T06066	*	4	X	X	X	ROM 83692	Guyana	Mazaruni River
<i>Paulasquama callis</i>	T06189	* †	5	X	X	X	ROM 83784	Guyana	Mazaruni River
<i>Pseudolithoxus anthrax</i>	T09934		5	X	X	X	AUM 53557	Venezuela	Caura River
<i>Pseudolithoxus anthrax</i>	T09282	*	5	X	X	X	AUM 53520	Venezuela	Orinoco River
<i>Pseudolithoxus anthrax</i>	V055		5	X	X	X	AUM 39246	Venezuela	Ventuari River
<i>Pseudolithoxus dumus</i>	T09512	*	5	X	X	X	ANSP 190757	Venezuela	Ventuari River
<i>Pseudolithoxus kelsorum</i>	T09895	*	5	X	X	X	AUM 51644	Venezuela	Orinoco River
<i>Pseudolithoxus nicoi</i>	P4647	*	5	X	X	X	AUM 43726	Venezuela	Casiquiare River
<i>Pseudolithoxus tigris</i>	T09376	* †	5	X	X	X	AUM 57674	Venezuela	Orinoco River
<i>Soromonichthys stearleyi</i>	V5533	* †	5	X	X	X	AUM 43872	Venezuela	Soromoni River
Pseudancistrus Clade									
<i>Pseudancistrus barbatus</i>	85.1	* †	5	X	X	X	MHNG 2653.059	French Guiana	Maroni River
<i>Pseudancistrus corantijnensis</i>	JMB1	*	5	X	X	X	MHNG 2672.092	French Guiana	Corentyne River
<i>Pseudancistrus depressus</i>	JMB2	*	5	X	X	X	MHNG 2651.069	Guyana	Essequibo River
<i>Pseudancistrus n.sp. Branco</i>	T14764		4	X	X	X	MCP 46103	Brazil	Branco River
<i>Pseudancistrus n.sp. Negro</i>	T14760		3	X	X	X	MCP 46144	Brazil	Negro River
<i>Pseudancistrus n.sp. Xingu L067</i>	85.2		4	X	X	X	MHNG 2586.046	Brazil	Xingu River
<i>Pseudancistrus n.sp. Xingu L067</i>	B1509	*	5	X	X	X	ANSP 199533	Brazil	Xingu River
<i>Pseudancistrus nigrescens</i>	85.3	*	5	X	X	X	MHNG 2651.069	Guyana	Essequibo River
<i>Pseudancistrus nigrescens</i>	G5942	*	5	X	X	X	AUM 45299	Guyana	Essequibo River
Lithoxus Clade									
<i>Exastilithoxus fimbriatus</i>	V049	* †	5	X	X	X	AUM 36632	Venezuela	Caroni River
<i>Exastilithoxus n.sp. Cuaio</i>	T09165	*	4	X	X	X	AUM 56685	Venezuela	Cuaio River
<i>Exastilithoxus n.sp. Iguapo</i>	V5561	*	4	X	X	X	AUM 43923	Venezuela	Iguapo River
<i>Exastilithoxus n.sp. Soromoni</i>	V5536	*	4	X	X	X	AUM 43875	Venezuela	Soromoni River
<i>Exastilithoxus n.sp. Ventuari</i>	T09667	*	5	X	X	X	AUM 54450	Venezuela	Ventuari River
<i>Lithoxus cf. stocki</i>	6909	*	5	X	X	X	ANSP 189135	Suriname	Maroni River
<i>Lithoxus jantjue</i>	T9020		5	X	X	X	AUM 39475	Venezuela	Ventuari River
<i>Lithoxus lithoides</i>	T412	†	4	X	X	X	AUM 37922	Guyana	Essequibo River
<i>Lithoxus pallidimaculatus</i>	T9021		5	X	X	X	AUM 50410	Suriname	Maroni River
<i>Lithoxus planquettei</i>	T9040		2	X			missing	French Guiana	Oyapock River
'Pseudancistrus' Clade									
<i>'Pseudancistrus' pectegenitor</i>	T09465	*	5	X	X	X	ANSP 190755	Venezuela	Ventuari River
<i>'Pseudancistrus' pectegenitor</i>	T09500	*	5	X	X	X	ROM 93342	Venezuela	Ventuari River
<i>'Pseudancistrus' sidereus</i>	T09506	*	5	X	X	X	ANSP 190756	Venezuela	Ventuari River
<i>'Pseudancistrus' sidereus</i>	T09532	*	5	X	X	X	AUM 54310	Venezuela	Ventuari River
Acanthicus Clade									
<i>Acanthicus adonis</i>	T9001		5	X	X	X	AUM 44605		aquarium specimen
<i>Acanthicus hystrix</i>	T9003	†	5	X	X	X	missing	Venezuela	Orinoco River
<i>Leporacanthicus galaxias</i>	V5427	†	5	X	X	X	AUM 42144	Venezuela	Ventuari River
<i>Leporacanthicus heterodon</i>	B2082		4	X	X	X	ANSP 193009	Brazil	Xingu River
<i>Leporacanthicus triactis</i>	T09826	*	5	X	X	X	AUM 54030	Venezuela	Ventuari River
<i>Megalancistrus parananus</i>	T14752	†	4	X	X	X	MCP 37991	Brazil	Paraná River
<i>Pseudacanthicus leopardus</i>	G5089	*	5	X	X	X	AUM 44440	Guyana	Essequibo River
<i>Pseudacanthicus n.sp. Xingu L025</i>	B2109		4	X	X	X	ANSP 193003	Brazil	Xingu River

<i>Hemiancistrus</i>											
<i>'Baryancistrus' beggini</i>	T09392	*	5	X	X	X	X	X	AUM 54990	Venezuela	Orinoco River
<i>'Baryancistrus' demantoides</i>	T09361	*	5	X	X	X	X	X	ROM 93339	Venezuela	Ventuari River
<i>'Hemiancistrus' guahiborum</i>	V096		3	X	X	X	X	X	AUM 39239	Venezuela	Ventuari River
<i>'Hemiancistrus' subviridis</i>	T09437	*	5	X	X	X	X	X	AUM 54456	Venezuela	Ventuari River
<i>Baryancistrus chrysolomus</i>	B1505	*	4	X	X	X	X	X	missing	Brazil	Xingu River
<i>Baryancistrus niveatus</i>	HLF1288	†	5	X	X	X	X	X	missing	Brazil	Iri River
<i>Baryancistrus xanthellus</i>	B1490	*	5	X	X	X	X	X	ANSP 199528	Brazil	Xingu River
<i>Hemiancistrus medians</i>	6948	*	†	5	X	X	X	X	ANSP 187122	Suriname	Maroni River
<i>Panaque armbrusteri</i>	B2189		4	X	X	X	X	X	ANSP 193093	Brazil	Xingu River
<i>Panaque bathyphilus</i>	P6269		5	X	X	X	X	X	AUM 45503	Peru	Marañon River
<i>Panaque cochliodon</i>	T14628	*	5	X	X	X	X	X	uncataloged	Colombia	Magdalena River
<i>Panaque nigrolineatus</i>	T09018	*	†	5	X	X	X	X	AUM 53764	Venezuela	Apure River
<i>Panaque schaeferi</i>	T9023	*	5	X	X	X	X	X	INHS 55408	Peru	Solimões River
<i>Parancistrus nudiventris</i>	B1526	*	5	X	X	X	X	X	ANSP 199530	Brazil	Xingu River
<i>Spectracanthicus punctatissimus</i>	B1496	†	5	X	X	X	X	X	ANSP 199539	Brazil	Xingu River
<i>Spectracanthicus zuanoni</i>	B1982		4	X	X	X	X	X	ANSP 199619	Brazil	Xingu River
<i>Hypostomini</i>											
<i>'Hemiancistrus' aspidolepis</i>	Genbank		1	X					Genbank		
<i>'Hemiancistrus' fuliginosus</i>	T14768		3	X	X	X			MCP 40028	Brazil	Saudade River
<i>'Hemiancistrus' maracaiboensis</i>	Genbank		1	X					Genbank		
<i>'Hemiancistrus' meizospilos</i>	T14750		4	X	X	X	X	X	MCP 40168	Brazil	Chapecó River
<i>'Hemiancistrus' punctulatus</i>	T14754		3	X	X	X	X	X	MCP 40946	Brazil	Carreiro River
<i>'Hemiancistrus' votuoro</i>	T14766		3	X	X	X			MCP 44181	Brazil	Passo Fundo River
<i>'Hypostomus' nigromaculatus</i>	Genbank		1	X					Genbank		
<i>Hypostomus (Coch.) hondae</i>	T07038		1	X					Genbank		
<i>Hypostomus (Coch.) macushi</i>	Genbank	*	5	X	X	X	X	X	ROM 85939	Guyana	Essequibo River
<i>Hypostomus (Coch.) plecostomoides</i>	Genbank		1	X					Genbank		
<i>Hypostomus (Coch.) pyrineusi</i>	T10377		5	X	X	X	X	X	AUM 51394	Peru	Madre de Dios River
<i>Hypostomus (Coch.) taphorni</i>	T07074	*	5	X	X	X	X	X	ROM 86352	Guyana	Essequibo River
<i>Hypostomus (Hyp.) boulengeri</i>	Genbank		3	X	X	X			Genbank		
<i>Hypostomus (Hyp.) commersoni</i>	Genbank		3	X	X	X			Genbank		
<i>Hypostomus (Hyp.) rhanthos</i>	T09530	*	5	X	X	X	X	X	AUM 54306	Venezuela	Ventuari River
<i>Hypostomus (Hyp.) robinii</i>	Genbank		1	X					Genbank		
<i>Hypostomus (Hyp.)</i> sp. Madre de Dios	T10282		5	X	X	X	X	X	AUM 51404	Peru	Madre de Dios River
<i>Hypostomus (Hyp.)</i> sp. Xingu	B1475		4	X	X	X	X	X	ANSP 199690	Brazil	Xingu River
<i>Pterygoplichthys disjunctivus</i>	Genbank		1	X					Genbank		
<i>Pterygoplichthys gibbiceps</i>	P4893		5	X	X	X	X	X	AUM 42131	Venezuela	Casiquiare River
<i>Pterygoplichthys multiradiatus</i>	Genbank		3	X	X	X			Genbank		
<i>Peckoltia</i> Clade											
<i>'Hemiancistrus' landoni</i>	T13836	*	5	X	X	X	X	X	AUM 93738	Ecuador	Clara River
<i>'Hemiancistrus' n.sp. L127</i>	T09143		5	X	X	X	X	X	ANSP 190894	Venezuela	Orinoco River
<i>'Hemiancistrus' pankimpuju</i>	P6233	*	4	X	X	X	X	X	AUM 45595	Peru	Marañon River
<i>'Panaqolus' koko</i>	108.1	*	4	X	X	X	X	X	MNHN 2011-0013	French Guiana	Maroni River
<i>'Peckoltia' feldbergae</i>	B2178		5	X	X	X	X	X	ANSP 193088	Brazil	Bacaja River
<i>'Peckoltia' feldbergae</i>	B2072		5	X	X	X	X	X	ANSP 193012	Brazil	Iri River
<i>'Spectracanthicus' 'immaculatus</i>	T1385	*	5	X	X	X	X	X	ANSP 194670	Brazil	Xingu River (mouth)
<i>'Spectracanthicus' 'immaculatus</i>	T1387	*	4	X	X	X	X	X	ANSP 194670	Brazil	Xingu River (mouth)
<i>Aphanotorulus ammophilus</i>	Genbank		1	X					Genbank		
<i>Aphanotorulus emarginatus</i>	B2046		4	X	X	X	X	X	ANSP 199645	Brazil	Xingu River
<i>Aphanotorulus squalinus</i>	T09528		5	X	X	X	X	X	AUM 54305	Venezuela	Ventuari River
<i>Etsaputu relictum</i>	CH157	†	5	X	X	X	X	X	MUSM 44256	Peru	Huallaga River
<i>Etsaputu relictum</i>	P6099	*	†	5	X	X	X	X	AUM 45531	Peru	Marañon River
<i>Hypancistrus contradens</i>	T09355	*	5	X	X	X	X	X	ANSP 190815	Venezuela	Ventuari River
<i>Hypancistrus debilitera</i>	T09279	*	5	X	X	X	X	X	AUM 53528	Venezuela	Orinoco River
<i>Hypancistrus furunculus</i>	V028	*	5	X	X	X	X	X	AUM 39225	Venezuela	Orinoco River
<i>Hypancistrus lunaorum</i>	T09562	*	5	X	X	X	X	X	ROM 92224	Venezuela	Ventuari River
<i>Hypancistrus</i> n.sp. Xingu L174	B2141		5	X	X	X	X	X	ANSP 193084	Brazil	Xingu River
<i>Isorineloricaria spinosissima</i>	T13692	*	†	5	X	X	X	X	ROM 93722	Ecuador	Guayas River
<i>Micracanthicus vandragti</i>	T09490	*	†	5	X	X	X	X	ANSP 190780	Venezuela	Ventuari River
<i>Panaqolus albomaculatus</i>	P6121		5	X	X	X	X	X	AUM 45502	Peru	Marañon River
<i>Panaqolus gnomus</i>	P6128	*	†	5	X	X	X	X	AUM 45501	Peru	Marañon River
<i>Panaqolus maccus</i>	T09009	*	5	X	X	X	X	X	AUM 53768	Venezuela	Guanare River
<i>Panaqolus</i> n.sp. Tacutu L306	G5183	*	5	X	X	X	X	X	AUM 44721	Guyana	Tacutu River
<i>Panaqolus nocturnus</i>	P6126	*	4	X	X	X	X	X	AUM 45500	Peru	Marañon River
<i>Peckoltia furcata</i>	P6200		5	X	X	X	X	X	AUM 45593	Peru	Marañon River
<i>Peckoltia</i> aff. vittata	T09533		5	X	X	X	X	X	AUM 54314	Venezuela	Orinoco River
<i>Peckoltia braueri</i>	T06465	*	5	X	X	X	X	X	ROM 86240	Guyana	Takutu River
<i>Peckoltia compta</i>	T10775		5	X	X	X	X	X	ROM 91263	Brazil	Tapajós River
<i>Peckoltia lineola</i>	T09831	*	5	X	X	X	X	X	AUM 54033	Venezuela	Ventuari River
<i>Peckoltia</i> n.sp. Madeira L210	T14753		2	X					MCP 35628	Brazil	Madeira River
<i>Peckoltia sabaji</i>	T09602		5	X	X	X	X	X	ANSP 191152	Venezuela	Orinoco River
<i>Peckoltia sabaji</i>	B1969		5	X	X	X	X	X	ANSP 199615	Brazil	Xingu River
<i>Peckoltia vittata</i>	10514	†	3	X	X				missing	Brazil	Madeira River
<i>Peckoltia vittata</i>	B2152	*	†	5	X	X	X	X	ANSP 193078	Brazil	Xingu River
<i>Peckoltichthys bachi</i>	P6254	†	5	X	X	X	X	X	AUM 45592	Peru	Marañon River
<i>Scobinancistrus</i> aff. <i>pariolispos</i> L082	B2113		5	X	X	X	X	X	ANSP 193045	Brazil	Xingu River
<i>Scobinancistrus aureatus</i>	B2193		4	X	X	X	X	X	ANSP 193094	Brazil	Xingu River
<i>Scobinancistrus pariolispos</i>	B2088	†	4	X	X	X	X	X	ANSP 193006	Brazil	Xingu River

exclusive of the Paraná River species *Megalancistrus parananus*, received strong support (Node 80: BI: 1.0, ML: 100).

3.2.7. The *Hemiancistrus* Clade Fig. 4 and Table 2

The *Hemiancistrus* Clade, containing the genera *Baryancistrus*, *Hemiancistrus*, *Oligancistrus*, *Parancistrus*, and *Panaque*, was only weakly but consistently supported as monophyletic (Node 73: BI: 0.70, ML: 59). Two major subclades were moderately supported as monophyletic: the wood-eating genus *Panaque* (Node 72: BI: 1.0, ML: 72), and a clade containing all other genera (Node 68: BI: 1.0, ML: 59). All non wood-eating genera (i.e., *Baryancistrus*, *Hemiancistrus*, *Oligancistrus*, *Parancistrus*) have historically been highly problematic from a taxonomic perspective and difficult to diagnose (e.g., Lujan et al., 2009). *Hemiancistrus* in particular has long been recognized as paraphyletic and treated as a repository for species that are difficult to place elsewhere. This is only the second phylogenetic study to examine the type species *H. medians* (the first being Covain and Fisch-Muller, 2012), which was not found to be closely related to any other nominal '*Hemiancistrus*' species in our study, suggesting that the genus may be monotypic. *Baryancistrus* was strongly supported as monophyletic (Node 60: BI: 1.0, ML: 99) only with the exclusion of the upper Orinoco species '*B. beggini*' and '*B. demantoides*'. These latter two species formed a moderately supported upper Orinoco clade (Node 67: BI: 1.0, ML: 73) with the species '*Hemiancistrus guahiborum*' and '*H. subviridis*'.

3.2.8. Tribe Hypostomini Fig. 4 and Table 2

We found the tribe Hypostomini to be well-supported as monophyletic (Node 57: BI: 1.0, ML: 88), and to significantly differ in composition from previous morphology-based analyses (Fig. 1B) despite still comprising mostly the species-rich genus *Hypostomus*. We found the geographically widespread genus *Pterygoplichthys* to be monophyletic (Node 56: BI: 1.0, ML: 100) and sister to a clade containing *Hypostomus* and the '*Hemiancistrus aspidolepis*' group (Node 54: BI: 1.0, ML: 97). Within the latter clade, only the genus *Hypostomus* received consistent support for monophyly (Node 48: BI: 1.0, ML: 91), with the BI analysis finding the northern and southern clades of '*He. aspidolepis*' group forming a polytomy with *Hypostomus* (Node 54). Respective northern and southern clades were resolved as monophyletic by both analyses: the Panamanian species '*He. aspidolepis*' and Venezuelan species '*He. maracaiboensis*' were found to be sisters (Node 52: BI: 1.0, ML: 98), and a southeastern Brazilian clade of all other species was found to be monophyletic (Node 51: BI: 1.0, ML: 100). However, only ML found these clades to be reciprocally monophyletic (Node 53: ML: 73; Supplemental Fig. 1).

Within *Hypostomus*, wood-eating species in the '*Hypostomus cochliodon* group' (also known as genus or subgenus *Cochliodon*) were found to be strongly monophyletic (Node 46: BI: 1.0, ML: 93) and to be well-supported as sister (Node 47: BI: 0.99, ML: 79) to a moderately supported clade of more stereotypically algivorous–detritivorous (i.e., having larger numbers of smaller teeth and generally straighter tooth rows) species restricted to northern South America (i.e., Amazon Basin and northward; Node 42: BI: 1.00, ML: 85). This topology for *Hypostomus* excludes the sole Paraná Basin species in our analysis ('*Hypostomus nigromaculatus*'), which is consistent with previous research showing that the southern South American '*Hypostomus*' species represent a geographically restricted radiation that is reciprocally monophyletic with respect to all other *Hypostomus* (Montoya-Burgos et al., 1998, 2002; Cardoso et al., 2012).

3.2.9. The *Peckoltia* Clade Fig. 4 and Table 2

The *Peckoltia* Clade is the most genus-rich tribe-level clade in our analysis, containing the genera *Aphanotorulus*, *Etsaputu*,

Hypancistrus, *Isorineloricaria*, *Micracanthicus*, *Panaqolus*, *Peckoltia*, *Peckoltichthys*, *Scobinancistrus*, and several species without clear generic affiliations (i.e., '*Hemiancistrus landoni*', '*Peckoltia feldbergae*', '*Panaqolus koko*', and '*Spectracanthicus immaculatus*'). Monophyly of the *Peckoltia* Clade inclusive of the Pacific-slope species '*Hemiancistrus landoni*' was weakly supported (Node 37: BI: 0.73, ML: 52), but monophyly of all *Peckoltia* Clade taxa exclusive of '*He. landoni*' was strongly supported (Node 36: BI: 1.0, ML: 95). Four apparently monotypic genera, including '*He. landoni*', were found near the base of the *Peckoltia* Clade. The Pacific-slope species *Isorineloricaria spinosissima*, which is sympatric with '*He. landoni*' in parts of the Pacific Coast of Ecuador, was weakly supported as sister to '*Spectracanthicus immaculatus*', which is from main channels of the lower Amazon Basin (Node 32: BI: 0.59, ML: –). *Peckoltichthys bachi*, which is restricted to tributaries of the upper Amazon River, was moderately supported as sister to a large clade of other widespread genera (Node 30: BI: 1.0, ML: 87). All other genera represented by more than one species or individual were moderately to strongly supported as monophyletic. These included *Aphanotorulus*, *Etsaputu*, *Hypancistrus*, *Peckoltia*, '*Peckoltia feldbergae*', *Panaqolus*, and *Scobinancistrus* (respective Nodes 35, 3, 28, 13, 20, 17, 19: BI: 1.0, ML: >80). Interestingly, monophyly of the wood-eating genus *Panaqolus* was only strongly supported (Node 17: BI: 1.0, ML: 97) with the exclusion of '*Panaqolus koko*', a curious species recently described from French Guiana with morphological characteristics apparently intermediate between *Panaqolus* and *Peckoltia* (Fisch-Muller et al., 2012). The ML analysis found weak support for '*Panaqolus koko*' to be sister to a clade containing *Etsaputu*, *Peckoltia*, *Panaqolus*, *Scobinancistrus*, and '*Peckoltia feldbergae*' (Node 23, ML: 56; Supplemental Fig. 1), whereas the BI analysis found these taxa to be monophyletic (BI: 1.0) but did not resolve many relationships among them.

Two recently described, monotypic genera (*Etsaputu* and *Micracanthicus*) were respectively nested within the genera *Peckoltia* and *Hypancistrus*, raising doubt as to their validity. The genus *Peckoltia* itself, a historically problematic taxon often confused with *Hemiancistrus* (Armbruster, 2004a, 2008), was well supported as monophyletic (Node 13: BI: 1.0, ML: 81), even as three putative populations of the type species *P. vittata* were found to be paraphyletic.

3.3. Macroevolutionary hypotheses

Our rearranged Hypostominae phylogeny no longer supported either the Trans-Highland Clade or the Diphyletic Wood-Eaters hypothesis. Instead of being nested within a clade of Ancistrini taxa restricted to the Guiana Shield, the predominantly Andean *Chaetostoma* Clade was found to be sister to all other Hypostominae. Many genera throughout the Hypostominae, and three whole tribe-level clades (i.e., the *Lithoxus* Clade, *Pseudancistrus* Clade, and '*Pseudancistrus*' Clade), have geographic ranges largely or entirely restricted to the Guiana Shield, suggesting that this area of northern South America may have been an early center of diversification. However, sister lineages successively removed from Hypostominae (i.e., Hypoptomatinae, Loricariinae, Rhinelepinae) are widespread throughout tropical South America, providing few clues as to the particular geographic origin of the *Chaetostoma* Clade. Our analysis also removed support for the Diphyletic Wood-Eaters hypothesis by finding no less than three independent origins for wood-eating in Hypostominae. Instead of a sister relationship between *Panaqolus* and *Panaque*, these genera were found to be members of different tribe-level clades, illustrating the likely morphological convergence that has occurred between these lineages and the *Cochliodon* clade within *Hypostomus* (Lujan and Armbruster, 2012).

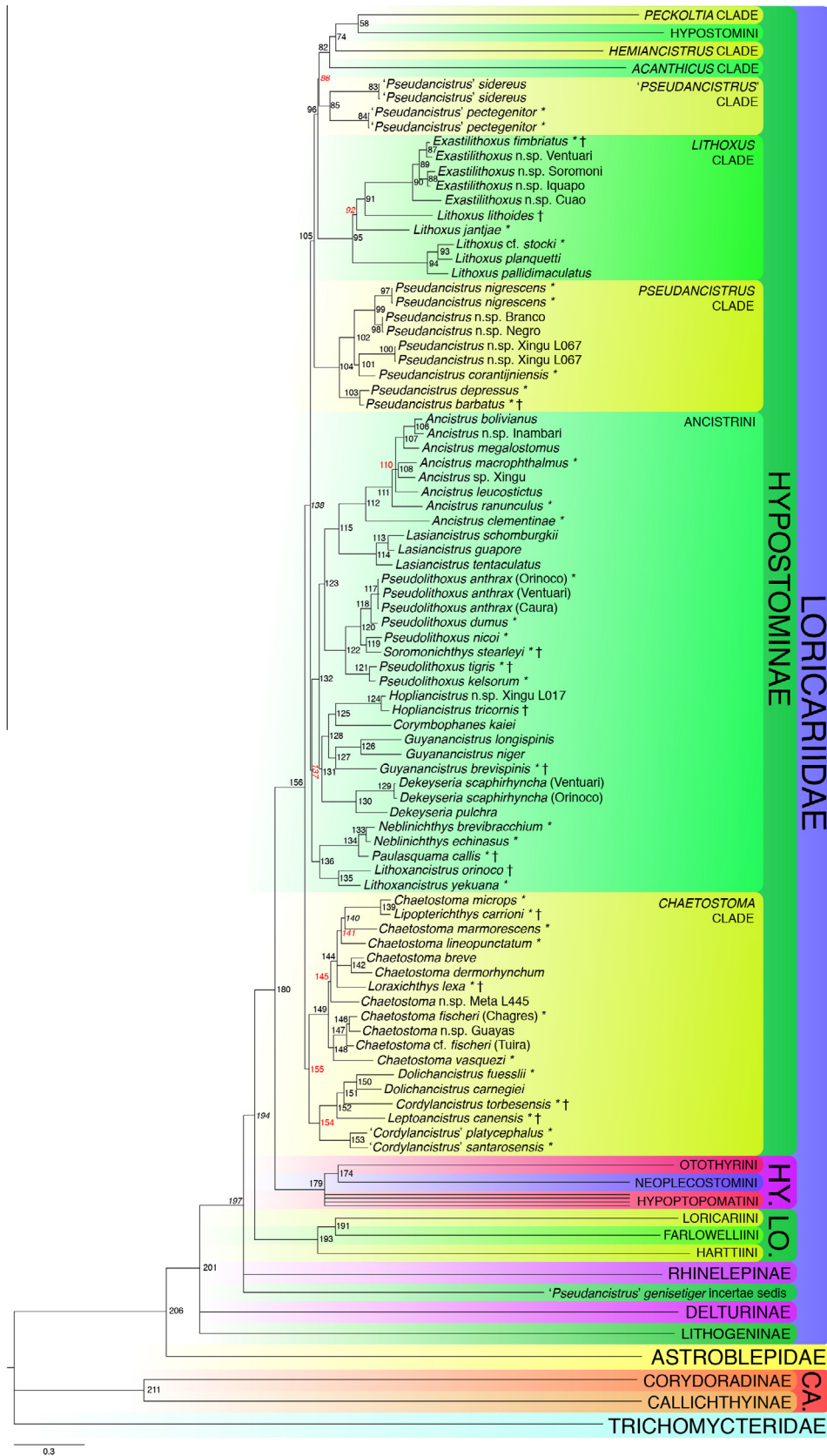


Fig. 3. Phylogenetic relationships of basal clades in subfamily Hypostominae expanded from Fig. 2. Node numbers correspond to Bayesian posterior probability (BI) and maximum likelihood (ML) support values in Table 2. Numbers in red indicate BI < 0.90; numbers in italics indicate ML < 50. Samples taken from at or near the type locality for a given species are indicated by asterisks (*) and species that are types for their genus are indicated by crosses (†). Relationships among nested clades in the Hypostominae are expanded in Fig. 4, with background color corresponding to each clade remaining unchanged. Abbreviations: CA. = Callichthyidae, HY. = Hypoptopomatinae, LO. = Loricariinae.

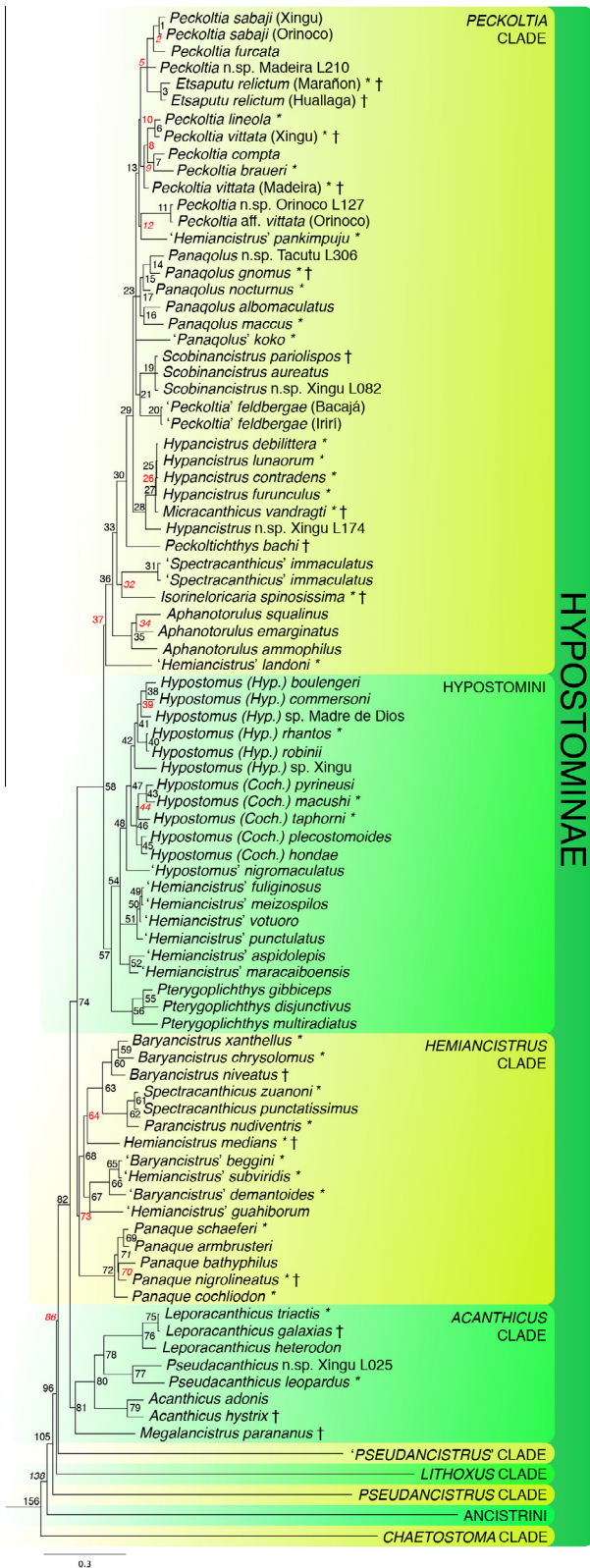


Fig. 4. Phylogenetic relationships of nested clades in subfamily Hypostominae expanded from Figs. 2 and 3. Node numbers correspond to Bayesian posterior probability (BI) and maximum likelihood (ML) support values in Table 2. Numbers in red indicate BI < 0.90; numbers in italics indicate ML < 50. Samples taken from at or near the type locality for a given species are indicated by asterisks (*) and species that are types for their genus are indicated by crosses (†). Abbreviations: Coch. = *Cochliodon*, Hyp. = *Hypostomus*.

4. Discussion

At the most inclusive taxonomic and phylogenetic scale, our study supported the reciprocal monophyly of Loricariidae and Astroblepidae, which has been a mostly consistent finding of every phylogenetic study since the first cladistic analysis of the Loricarioidei by Howes (1983). The only major difference between our results and those of some previous studies was in the placement of *Lithogenes* in Loricariidae (sensu Schaefer, 2003) vs. Astroblepidae (sensu Armbruster, 2004a, 2008; Hardman, 2005; Fig. 1B).

Our results support the monophyly of three large subfamilies that include the vast majority of loricariid species and are mostly congruent with previous morphology-based taxonomic delimitations of the Loricariinae, Hypoptopomatinae and Hypostominae (respective Nodes 193, 179, 156: BI: >0.95, ML: >70). However, relationships within the Hypostominae differ from those of previous morphology-based studies at many internal nodes, with many of the taxa in our study being examined here for the first time in a molecular phylogenetic context. We review the species richness of these clades, discuss some of their interesting biogeographical and ecomorphological characteristics, and compare our results with those of previous studies.

4.1. Reappraisal of Loricariidae subfamilies and tribes

4.1.1. Basal nodes

We found four relatively species-poor clades branching off at or near the base of the Loricariidae (Fig. 2): Lithogeninae (representing a total of 3 described spp.), Delturinae (7 spp.), Rhinelepinae (6 spp.), and *Pseudancistrus* *genisetiger* (2 spp.). All of these clades have relatively generalized trophic morphologies and diets; however, they are biogeographically intriguing because of their phylogenetic placement and their distributions across drainages that are mostly peripheral to the Amazon Basin. The Lithogeninae, for example, are restricted to the Guiana Shield and Coastal mountain ranges of Venezuela and Guyana, whereas the Delturinae are restricted to Atlantic coastal streams of southeastern Brazil (Reis et al., 2006). Recent morphological analyses (Fig. 1B, Armbruster, 2004a, 2008) have consistently found the Delturinae to be monophyletic and sister to all other Loricariidae, which, ignoring *Lithogenes*, was the same relationship found herein. Previous molecular studies that have sampled broadly across loricariid subfamilies (e.g., Montoya-Burgos et al., 1998; Chiachio et al., 2008) have likewise found Delturinae to be sister to all other Loricariidae.

Five of the six species in Rhinelepinae are restricted to southeastern Brazil, northern Argentina, Paraguay and Uruguay, with only one species (*Pseudorinelepis genibarbus*) distributed more broadly across the southern and western Amazon Basin. The placement of Rhinelepinae in Loricariidae has fluctuated across morphological and molecular analyses. We found strong BI support for Rhinelepinae to be monophyletic and in a polytomy with *Pseudancistrus* *genisetiger* and the clade of ((Hypostominae + Hypoptopomatinae) Loricariinae), whereas previous molecular studies have found it to be either sister to Loricariinae (Montoya-Burgos et al., 1998) or sister to Hypostominae + Hypoptopomatinae exclusive of Loricariinae (Cramer et al., 2011). In the first morphology-based phylogenetic analysis of the Rhinelepinae, Schaefer (1986) found the group to be paraphyletic, with *Pseudorinelepis* and *Pogonopoma* (then misidentified as *Pogonopomoides*) occurring at two different places in his phylogeny. Armbruster (1998) presented strong morphological support for monophyly of the Rhinelepinae, and in subsequent studies (2004a, 2008, Fig. 1B) found it to be sister to almost all other Hypostominae,

although relationships of the group to other hypostomines were variable and only weakly supported. It seems clear from both molecular and morphological data that Rhinelepineae is a distinct subfamily, although more data are needed to robustly establish its phylogenetic position.

'*Pseudancistrus*' *genisetiger* is a curious taxon that also has a very limited range in Atlantic coastal streams draining north along the easternmost tip of South America between Fortaleza and Natal, Brazil. This species has never been examined using morphology-based phylogenetic methods and the sole previous molecular analysis (Covain and Fisch-Muller, 2012) found it to be sister to *Hemipsilichthys* (Delturinae) and part of a clade with *Harttia* (Loricariinae) that was sister to all other Loricariidae. However, that study had limited taxonomic sampling across loricariid subfamilies. Regardless of the poor and inconsistent support for placement of '*Pseudancistrus*' *genisetiger*, it seems likely that this species represents not only a new genus, but likely also a new subfamily along with the sympatric '*Pseudancistrus*' *papariae*.

4.1.2. Subfamily Loricariinae

The subfamily Loricariinae contains approximately 220 described species distributed throughout most of the geographic range of the Loricariidae. The Loricariinae are distinguished by having highly dorsoventrally depressed and elongate bodies with long, thin caudal peduncles, and often highly reduced jaw structures. The morphology-based phylogenetic analysis of Rapp Py-Daniel (1997) was the first to examine all existing Loricariinae genera, which she resolved into two tribes: Loricariini and Harttiini. Composition of these tribes has since expanded and their inter-generic relationships reexamined by four morphological and eight molecular analyses (including this study). Results of the morphological study by Armbruster (2004a) paralleled those of Rapp Py-Daniel (1997), but subsequent morphological studies have found only Loricariini to be monophyletic, with Harttiini being either a paraphyletic group successively removed from Loricariini (de Paixão and Toledo-Piza, 2009) or being distributed across an unresolved polytomy inclusive of Loricariini (Provenzano, 2011). Our analysis parallels these morphological and other previous molecular studies that have found only Loricariini to be monophyletic, with only those Harttiini genera exclusive of *Harttia* being monophyletic and sister to Loricariini, and *Harttia* being sister to all other Loricariinae (Montoya-Burgos et al., 1998; Hardman, 2005; Sullivan et al., 2006; Covain et al., 2008; Rodriguez et al., 2011).

4.1.3. Subfamily Hypoptopomatinae

The subfamily Hypoptopomatinae is a clade of approximately 85 small-bodied (<11 cm SL) species that are mostly restricted to southeastern Brazil, with only a few genera distributed across Atlantic-slope drainages north of the Amazon River. The morphology-based phylogenetic analysis of Schaefer (1991) was the first to examine all existing Hypoptopomatinae genera, which it resolved into the two tribes Hypoptopomatini and Othothyriini, with *Neoplecostomus* being an outgroup. The monophyly and sister relationship of these tribes exclusive of the Neoplecostominae has since received additional morphological support from Rapp Py-Daniel (1997) and Schaefer (1998). Armbruster (2004a) expanded the Neoplecostominae to include *Isbrueckerichthys*, *Kronichthys*, *Pareiorhaphis* (then *Hemipsilichthys*), and *Pareiorhina*, but noted that this Neoplecostominae was paraphyletic in his analysis with respect to the Hypoptopomatinae (Fig. 1B). This and other molecular studies have consistently recovered Neoplecostominae genera as monophyletic and nested within Hypoptopomatinae, either sister to the Hypoptopomatini (Montoya-Burgos et al., 1998) or the Othothyriini (Chiachio et al., 2008; Cramer et al., 2011, this study).

4.2. Reappraisal of Hypostominae tribes and genera

All other taxa in our study were resolved into nine tribe-level clades within the Hypostominae, and these will be discussed sequentially.

4.2.1. The *Chaetostoma* Clade

The predominantly Andean *Chaetostoma* Clade was found to be sister to all other Hypostominae. This clade contains 58 species that are mostly restricted to swift-flowing piedmont streams draining Atlantic and Pacific slopes of the Andes from Panama to southern Peru, with several species distributed along the northern coastal mountain ranges of Venezuela (including the Lake Valencia drainage), one species each in northern and southern drainages of the Guiana Shield, and one undescribed species in the Brazilian Shield. All *Chaetostoma* Clade species have broad jaws with long rows of many small teeth, and are relatively generalized grazers of algae and detritus. Our results support the recognition of four valid genera (*Chaetostoma*, *Cordylancistrus*, *Dolichancistrus*, *Leptoancistrus*), the invalidation of two genera (*Lipoptericthys*, *Loraxichthys*), and the erection of at least one new genus.

Our *Chaetostoma* Clade is congruent with the '*Chaetostoma* group' that Armbruster found to be strongly monophyletic (Fig. 1B) but nested within his much larger tribe Ancistrini. Like Armbruster (2004a, 2008), we found *Chaetostoma* to include the narrowly restricted, monotypic genus *Lipoptericthys* from southern Ecuador, as well as the recently described genus and species *Loraxichthys lexa* from central Peru. In contrast, Salcedo (2013) found *Lipoptericthys* and *Loraxichthys* to be part of a clade with the Panamanian genus *Leptoancistrus*. In our analysis, *Leptoancistrus* was well-supported as sister to two other northern Andean genera: *Dolichancistrus* and *Cordylancistrus* sensu stricto (i.e., the type species *Co. torbesensis*).

The genus *Cordylancistrus* has never undergone a taxonomic revision or been previously investigated from a molecular phylogenetic perspective, but morphological studies have consistently found it to be paraphyletic (Fig. 1B, Armbruster, 2008; Salcedo, 2013). Likewise, we found that the type species, *Co. torbesensis* (from an Atlantic-slope drainage in the southern Venezuelan Andes), is distantly related to '*Co.*' *platycephalus* (from Atlantic-slope drainages of the southern Ecuadorian Andes). The latter species, though, was well-supported as sister to the recently described species '*Co.*' *santarosensis* from drainages along the Pacific Coast of Ecuador (Tan and Armbruster, 2012). There is therefore strong morphological and molecular support for the erection of at least one new genus for the trans-Andean (i.e., Atlantic and Pacific slope) clade of '*Co.*' *platycephalus* + '*Co.*' *santarosensis*. There are also several more species not in our analysis that have been placed into *Cordylancistrus*; however, most of these, like '*Co.*' *platycephalus*, differ significantly from the type species. More data from more taxa are therefore needed to resolve relationships among these '*Cordylancistrus*' lineages.

Our results describe a biogeographical pattern for the *Chaetostoma* Clade in which a strongly monophyletic group of mostly northern Andean genera (*Cordylancistrus*, *Dolichancistrus*, and *Leptoancistrus*) is sister to the more widespread genus *Chaetostoma*. *Chaetostoma* includes the only species from this tribe-level clade that occur outside the Andes (i.e., *Ch. vasquezii* and *Ch. jegui* from respectively northern and southern drainages of the Guiana Shield, and an undescribed *Chaetostoma* species from the Brazilian Shield). Within *Chaetostoma*, a species-poor clade of northern Atlantic and Pacific slope species (*Ch.* n.sp. *Guayas*, *Ch. fischeri*, *Ch. vasquezii*) is sister to an entirely Atlantic-slope clade that ranges from northern Venezuela to southern Peru and the Brazilian Shield. The relationship of the geographically widespread Atlantic-slope clade to two successive sister groups restricted to northern South American

drainages, suggests that the widespread clade originated after Andean uplift and radiated from north to south along the headwaters of Orinoco and Amazon tributaries draining this mountain range; however, a time-calibrated analysis inclusive of more populations and species is needed to resolve this pattern more precisely and robustly.

4.2.2. Tribe Ancistrini

Ancistrini is the second most genus-rich tribe-level clade in our study, spanning 10 valid genera and considerable morphological diversity, including a seven-fold range in body size, from *Soromonichthys stearleyi* (3 cm SL), to *Dekeyseria scaphirhyncha* (>21 cm SL). The geographic range of this clade covers most of tropical northern South America. The tribe-level name ‘Ancistrini’ has historically applied much more broadly to most members of the Hypostominae having enlarged and highly evertible cheek odontodes (Isbrücker, 1980). Within this Ancistrini *sensu lato*, Armbruster (2008, Fig. 1B) found an ‘Ancistrus clade’ that is similar to the Ancistrini *sensu stricto* of this study. Armbruster’s (2008) Ancistrus clade had the same topology as this study for relationships among the genera *Ancistrus*, *Lasiancistrus*, *Pseudolithoxus*, *Hopliancistrus*, and *Neblinichthys* (Fig. 1B). However, that study excluded the genera *Soromonichthys*, *Corymbophanes*, *Guyanancistrus*, *Dekeyseria*, and *Lithoxancistrus* from the Ancistrus clade and did not consider genus *Paulasquama*, which was not yet described. The strong support that this study found for inclusion of these genera among members of the Ancistrus clade is without precedent. Indeed, the genera *Guyanancistrus* and *Lithoxancistrus*, which are recognized as valid and respectively monophyletic, were previously treated as junior synonyms of the genus *Pseudancistrus* (Armbruster, 2008). *Soromonichthys* had been found to be a monotypic genus closely related to the *Chaetostoma* and *Lithoxus* clades, but is here nested within genus *Pseudolithoxus*. And the genus *Corymbophanes* had been found to be its own tribe sister to all other Hypostominae, but is here nested within the Ancistrini and is well-supported as sister to *Hopliancistrus*.

The Ancistrini genus *Ancistrus* is among the most ubiquitous and geographically widespread of all loriciariid genera. It can be common in both relatively lentic lowland habitats and torrential mountain streams up to 1100 meters above sea level (e.g., *Ancistrus marcapatae*, Lujan et al., 2013). The genus can be distinguished from all other Loriciariidae by having a profusion of sexually dimorphic, fleshy, mucous-covered tentacles on the snouts of males, which may function to attract females to nest cavities (Sabaj et al., 1999). Our analysis included two of the most curious *Ancistrus* species, the Pacific-slope species *A. clementinae* from the Pacific Coast of Ecuador, and the strikingly flat and broad-headed species *A. ranunculus* from the Xingu River. *Ancistrus ranunculus* is morphologically convergent with the genus *Parancistrus* and they share an apparently specialized diet consisting of loosely aggregated, flocculent detritus (Zuanon, 1999). *Ancistrus clementinae* was found to be sister to an Atlantic-slope clade containing all other congeners, and *A. ranunculus* was found to be sister to all other congeners exclusive of *A. clementinae*.

4.2.3. The Pseudancistrus Clade

The *Pseudancistrus* Clade is generally distinguished by being dorsoventrally depressed, by having hypertrophied odontodes along the lateral margins of the snout (regardless of sex or season), and by having hypertrophied cheek odontodes that are evertible to less than 45° from the body (Isbrücker et al., 1988; Armbruster, 2004b, 2008). The *Pseudancistrus* Clade in this study differs from Armbruster’s (2004a,b, 2008; Armbruster and Taphorn, 2008) ‘*Pseudancistrus sensu stricto*’ clade by excluding ‘*Pseudancistrus genisetiger* and ‘*Ps. papariae*, but is consistent with the composition

and relationships of the *Pseudancistrus barbatus* clade as revealed by Covain and Fisch-Muller (2012) and Silva et al. (2014).

Four of the six described species in the *Pseudancistrus* Clade are restricted to Atlantic Coastal drainages of the Guianas (*Ps. barbatus*, *Ps. corantijiensis*, *Ps. depressus*, and *Ps. nigrescens*), while one putative member is from the eastern Orinoco basin (i.e., *Ps. reus*, Armbruster, 2008b, not examined here), and one described species (*Ps. zawadzki*, not examined here) is from the Tapajos River draining the northern Brazilian Shield. Undescribed species are known from northern (e.g., the Branco, Negro, and Trombetas rivers) and southern (Xingu Rivers) tributaries of the lower Amazon.

Intriguingly, we found the one undescribed species in our analysis from south of the Amazon River (*Ps. n.sp.* Xingu L067) to be sister to *Ps. corantijiensis*, exclusive of populations from the Negro, Branco (undescribed species), and Essequibo (*Ps. nigrescens*) rivers. This suggests that *Pseudancistrus* dispersed from north to south not via the largest modern main river channels (i.e., the Essequibo and Branco), but rather via headwater capture across the Acarai Mountain range that forms the border between southeastern Guyana and Brazil and gives rise to headwaters of the Courantyne River to the north and the Trombetas River to the south. A similar pattern of headwater dispersal between northern coastal and southern Amazon drainages in the eastern Guiana Shield has been hypothesized for species of the callichthyidae genus *Corydoras* (*C. bondi*, Nijssen, 1970) based on morphological data, and the Ancistrini genus *Guyanancistrus* (*G. brevispinis*, Cardoso and Montoya-Burgos, 2009) based on molecular data. However, further analyses of *Pseudancistrus* inclusive of populations from the intervening Trombetas River will be needed to resolve the historical biogeography of this group in greater detail.

4.2.4. The Lithoxus Clade

All three genera and ten described species in the *Lithoxus* Clade are geographically restricted to the Guiana Shield in northern South America. The clade is morphologically distinguished by having small (<7 cm SL), dorsoventrally depressed bodies, a large oral disk, and small clusters of elongate teeth corresponding to their invertivorous diet. The *Lithoxus* Clade has only previously been examined in a molecular phylogenetic framework by Covain and Fisch-Muller (2012), who examined only two *Lithoxus* species and found them to be monophyletic. Most of what is known about systematic relationships within the *Lithoxus* Clade is based on taxonomic research (Boeseman, 1982; Isbrücker and Nijssen, 1985; Lujan, 2008). Armbruster (2004a, 2008) examined two species of *Lithoxus* (*L. lithoides* and *L. bovallii*) and *Exastilithoxus fimbriatus* and found these to be well-supported as monophyletic within his Ancistrus clade (Fig. 1B). Of the ten species or populations that we examined, five lineages assignable to *Exastilithoxus* (including the type species *E. fimbriatus*) were well-supported as monophyletic and sister to the type species of *Lithoxus* (*L. lithoides*). A second species, *L. jantjæ*, was found to be sister to this clade, with the remaining three species forming a well-supported clade sister to all other species. The genus *Lithoxus* was therefore paraphyletic in our analysis, but the statistical support for relationships between both *L. lithoides*, *L. jantjæ* and other species was weak.

Our finding that the three eastern Guiana Shield species (*Lithoxus* cf. *stocki*, *L. planquettei*, and *L. pallidimaculatus*) were monophyletic lends support to a morphology-based hypothesis first proposed by Boeseman (1982; with further support from Lujan, 2008), that these species comprise a subgenus separate from the western Guiana Shield species *L. lithoides* and *L. jantjæ*. Boeseman (1982) erected the name *Paralithoxus* for the former, with the latter being retained in subgenus *Lithoxus* (Lujan, 2008); however, *Paralithoxus* was synonymized with *Lithoxus* by Nijssen and Isbrücker (1990) and we did not examine the type species of

Paralithoxus (*Lithoxus bovallii*) leaving validity of these subgenera an open question.

4.2.5. The 'Pseudancistrus' Clade

The tribe-level 'Pseudancistrus' Clade represents the fifth and final remnant of taxa formerly recognized by Armbruster (2008) as part of his 'Pseudancistrus sensu lato' clade (Fig. 1B). It contains two species ('Ps.' *pectegenitor* and 'Ps.' *sidereus*) that were found to be paraphyletic in the morphological analysis of Armbruster (2008, Fig. 1B), but are well-supported as monophyletic herein. Both species are geographically restricted to main channels of the upper Orinoco and Casiquiare rivers and lower courses of their tributaries. The only previous molecular phylogeny to examine these taxa is that of Covain and Fisch-Muller (2012), who found this clade to be sister to *Lithoxus*, although their study examined relatively few additional taxa.

4.2.6. The Acanthicus Clade

The tribe-level *Acanthicus* Clade contains four genera and 14 species that are distributed across nearly the entire Atlantic-slope range of the Loricariidae. The *Acanthicus* Clade is largely restricted to main river channel habitats where they exhibit a wide range of trophic specializations, from macroinvertebrate probers (*Leporacanthicus* and *Pseudacanthicus*), to spongivores (*Megalancistrus*; Delariva and Agostinho, 2001), to big river algivores and detritivores (*Acanthicus*). The *Acanthicus* Clade also exhibits a nearly seven-fold range in body size, from *Leporacanthicus joselimae* (<10 cm SL) to *Acanthicus hystrix* (>63 cm SL), and is distinguished by having bodies covered in an abundance of short, stout, and sharp odontodes that give the clade its name.

All four *Acanthicus* Clade genera were also found to form a monophyletic group by Armbruster (2004a, 2008), although intergeneric relationships differed from this study (Fig. 1B). No previous molecular study has examined all four *Acanthicus* Clade genera, although several studies have examined two or three genera and have consistently found these taxa to form a monophyletic group (e.g., Montoya-Burgos et al., 1998; Hardman, 2005; Cramer et al., 2011).

4.2.7. The Hemiancistrus Clade

The tribe-level *Hemiancistrus* Clade contains six described genera and over 21 species distributed across the Amazon, Orinoco, Magdalena, Essequibo, and coastal Guiana Shield basins. Although most members of the *Hemiancistrus* Clade are herbivore-detritivores, the clade includes two intriguingly specialized genera: *Parancistrus*, which appears to be specialized for the consumption of flocculent detritus (Zuanon, 1999), and *Panaque*, which is specialized for the consumption of wood (Lujan et al., 2011; Lujan and Armbruster, 2012). The clade spans an almost 7.5-fold range in body size, from 'Baryancistrus' *beggini* (8 cm SL) to *Panaque schaeferi* (>60 cm SL).

Armbruster (2004a, 2008) recognized the genus *Hemiancistrus* as highly paraphyletic (Fig. 1B). However, he did not examine the type species *H. medians* until recently, when it was found to be part of a polytomy with many other 'Hemiancistrus' species (JWA, unpublished data). Our analysis suggests that *Hemiancistrus* may be monotypic, with *H. medians* being only distantly related to all other 'Hemiancistrus' in our analysis. Indeed, the generic composition and intergeneric relationships that we found for the *Hemiancistrus* Clade have little precedent, except that all were included together in Armbruster's (2004a, 2008) 'Panaque clade' (Fig. 1B).

We found *Panaque* to be well-supported as monophyletic, with the Magdalena River species *Pan. cochliodon* being sister to an entirely Atlantic-slope clade containing all other species. *Panaque* was weakly supported as sister to the clade containing all other genera, which was divided into two geographically restricted

clades: A strongly monophyletic clade of upper Orinoco species ('*Hemiancistrus*' *guahiborum*, '*H.*' *subviridis*, '*Baryancistrus*' *demantoides*, and '*B.*' *beggini*) was well-supported as sister to a strongly monophyletic clade of lower Amazon Basin species + *H. medians* (from the Atlantic Coastal Maroni River basin in Suriname). The lower Amazon Basin clade appears to represent a localized radiation limited to mostly clearwater Amazon tributaries draining the Guiana and Brazilian shields, with all specimens in this analysis coming from the Xingu River on the Brazilian Shield.

The weakness of morphological evidence supporting monophyly of *Baryancistrus* inclusive of '*B.*' *beggini* and '*B.*' *demantoides* was discussed by Lujan et al. (2009), but morphological data have been too limited to support the description of a new genus. Our results support a reexamination of this group, a narrower delimitation of *Baryancistrus*, and the erection of a new genus for the clade of upper Orinoco '*Baryancistrus*/*Hemiancistrus*'. Outside of the morphology-based studies of Armbruster (2004a, 2008), few other phylogenetic studies have examined members of the *Hemiancistrus* Clade and those that have included only one or two species (e.g., Montoya-Burgos et al., 1998; Cramer et al., 2011).

4.2.8. Tribe Hypostomini

Our tribe Hypostomini includes two described genera and approximately 170 species, making this the most species-rich tribe-level clade in the Hypostominae. The Hypostomini is among the most ubiquitous freshwater fish groups throughout tropical South America and includes two genera (*Hypostomus* and *Pterygoplichthys*) that have been the most problematic invasive loricariids outside their native range (e.g., Capps and Flecker, 2013). The vast majority of Hypostomini species are generalized detritivores. Indeed, Lujan et al. (2012) found that *Hypostomus* and *Pterygoplichthys* were consistently among the most depleted in ¹⁵N isotope relative to other sympatric loricariids, suggesting that they specialize on a particularly protein-poor diet. Among these detritivores and within *Hypostomus*, the *Cochliodon* clade is exceptional in its morphological specializations for the consumption of wood (Lujan et al., 2011; Lujan and Armbruster, 2012).

The Hypostomini group with the greatest morphological diversity are '*Hypostomus*' from the upper Paraná and Uruguay river systems in southeastern Brazil, Paraguay, Uruguay, and northern Argentina – regions that have a correspondingly low diversity of non-Hypostomini loricariids. Although our study includes only a single representative of this clade ('*Hypostomus*' *nigromaculatus*), our results are consistent with previous molecular phylogenetic studies that indicate that the Paraná '*Hypostomus*' are a monophyletic, geographically restricted radiation sister to all taxa in the Amazon Basin and northward (i.e., *Hypostomus* sensu stricto; Montoya-Burgos et al., 1998, 2002; Cardoso et al., 2012).

Armbruster (2004a, 2008) found morphological support for monophyly of the *Hypostomus* group (Fig. 1B); however, our results differ from his by combining his tribes Pterygoplichthyini with the Hypostomini, and by removing the genera *Isorineloricaria* and *Aphanotorulus*. Previous molecular studies have provided congruent support for monophyly of a clade that matches our Hypostomini, with parallel topologies of intertribe relationships and only a distant relationship between Hypostomini and *Isorineloricaria* + *Aphanotorulus* (Montoya-Burgos et al., 2002; Cramer et al., 2011; Cardoso et al., 2012).

4.2.9. The Peckoltia Clade

The *Peckoltia* Clade is morphologically diverse and the most genus-rich tribe-level clade in the Hypostominae. It encompasses nine described, valid genera (52 species), and has a geographic range covering much of northern South America. It also spans an over 12-fold range in body size, from *Micracanthicus vandragtii* (4 cm SL; Lujan and Armbruster, 2011a) to *Isorineloricaria spinosissimus*

(52 cm SL; K. Ray, pers. comm.). Although most members of the *Peckoltia* Clade are generalized algivore-detritivores, the clade includes the specialized wood-eating genus *Panaqolus* (Lujan et al., 2011; Lujan and Armbruster, 2012) and the specialized invertivorous genera *Hypancistrus* and *Scobinancistrus*.

Despite the morphological diversity of the *Peckoltia* Clade, its internal branches were mostly short and there were several poorly resolved or weakly supported relationships. Our study is the first to present strong support for monophyly of the genus *Peckoltia*, despite the fact that three putative populations of the type-species *P. vittata* were found to be paraphyletic. *Peckoltia vittata* was originally described based on a syntype series including specimens from the main channel of the lower Amazon near the Xingu River mouth, and the upper Amazon in the Madeira River, exclusive of the Orinoco River (Steindachner, 1881). Herein, we examined specimens from both these locations plus the upper Orinoco River, and none were found to be each other's closest relatives, indicating that a lectotype should be designated for *P. vittata* so that species-level systematics may be clarified.

The relationships that we recovered for the *Peckoltia* Clade have little precedent in the morphology-based studies of Armbruster (2004a, 2008), except that most taxa included in this clade were previously part of his 'Panaque clade'. The only previous molecular phylogenetic study to include more than just a few members of our *Peckoltia* Clade was that of Cramer et al. (2011), who also found strong statistical support for their monophyly but recovered a large polytomy for most internal relationships.

4.3. Biogeography

The rearranged topology of Hypostominae lineages that we inferred from molecular evidence removed support for the 'Trans-Highland Clade' hypothesis, but is suggestive of other biogeographical patterns and processes. Indeed, across the Hypostominae, there is now a strong signal of evolutionary diversification in rivers draining highlands of the Guiana Shield. Four tribes arising from relatively basal nodes, including seven of the 10 genera in Ancistrini, all but one member of the *Pseudancistrus* Clade, the entire *Lithoxus* Clade, and both members of the '*Pseudancistrus*' Clade are geographically restricted to rivers draining the Guiana Shield.

Today, Guiana Shield rivers are highly disconnected, comprising tributaries of the upper and lower Orinoco, the Negro, the Branco, the Essequibo, as well as the lower Amazon River and smaller rivers draining the northeastern coast of South America. Several geological studies (summarized in Lujan, 2008; Lujan and Armbruster, 2011b) suggest that until at least the early Pliocene (~5 Mya; McConnell, 1959; Gibbs and Barron, 1993) a major paleodrainage called the proto-Berbice united many headwaters and main channels of western Guiana Shield drainages in a single large watershed comparable in size to the modern Orinoco. The proto-Berbice is thought to have formed in the early Paleogene (~60 Mya), approximately coincident with the early diversification of the Loricariidae (Lundberg et al., 2007). The proto-Berbice may therefore help explain the currently highly disjunct distributions of many tribes across headwaters of several major drainage basins. Much finer resolution, time-calibrated molecular data will be needed, though, to reconstruct in greater detail the biogeographical relevance of the proto-Berbice to loricariid evolution.

5. Conclusions

Our study provides a new evolutionary understanding of the fifth most species-rich vertebrate family on Earth; however, many nodes remain unresolved or weakly supported and, with only

approximately 200 loricariid species (or one quarter of the over 800 described, valid loricariid species; Eschmeyer, 2014) in our analysis, there is much work to be done. Particularly species-rich genera with broad distributions and poorly resolved alpha taxonomy (e.g., *Ancistrus*, *Chaetostoma*, *Hypostomus*, *Peckoltia*) need the most attention, as do the relatively poorly resolved relationships among *Peckoltia* Group genera.

Future research should also focus on time-calibrating the loricariid phylogeny. Unfortunately, the fossil record for the Loricariidae is very scarce and relatively recent. Indeed, the only fossil that might be particularly helpful for calibrating any phylogeny for groups within the Loricarioidei is that of the late Paleocene *Corydoras revelatus* from the family Callichthyidae (Reis, 1998). Although problematic when used to try to test biogeographical hypotheses, time calibration via nodes that represent vicariant events likely caused by the well-dated acceleration in uplift of the Andes Mountains should also be considered. Our phylogeny, for example, includes eight Pacific-slope lineages from six tribes (Loricariini: *Rineloricaria jubata*; *Chaetostoma* Clade: '*Cordylancistrus*' *santarosensis*, *Chaetostoma* n.sp. Guayas; Ancistrini: *Ancistrus clementinae*; *Hemiancistrus* Clade: *Panaque cochliodon*; Hypostomini: *Hemiancistrus maracaiboensis*, *Hypostomus hondae*; *Peckoltia* Clade: '*Hemiancistrus*' *landoni*, *Isorineloricaria spinosissimus*). Assuming that late Miocene acceleration in uplift of the central and northern Andes Mountains (Gregory-Wodzicki, 2000) was the principal event causing the genetic isolation of these lineages from Atlantic-slope sister lineages, these constitute a source of calibration points that is too rich to ignore. Regardless, it is clear from the nested position of all these taxa that much of the diversification of the Loricariidae occurred well before major uplift of the Andes Mountains, which is now known to be the case for much of the Neotropical ichthyofauna (Albert et al., 2011).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympcv.2014.08.020>.

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